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| <b>(54) Title:</b> OLIGONUCLEOTIDES WITH ACTIVITY AGAINST HUMAN IMMUNODEFICIENCY VIRUS<br><br><b>(57) Abstract</b><br><br>The present invention features compounds and methods for inhibiting propagation of human immunodeficiency virus (HIV). Preferred HIV target sites are identified and oligonucleotides designed to hybridize to a target site are described. The preferred use of the oligonucleotides is as an anti-HIV agent to inhibit HIV propagation in a patient infected with HIV. Other uses of the present invention include detecting the presence of HIV by using the oligonucleotides as detection probes or amplification primers, and measuring the ability of an oligonucleotide to inhibit HIV propagation to evaluate its suitability as an anti-HIV agent for a phenotype of HIV or diagnose the presence of HIV in a patient. |           |   |

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DESCRIPTIONOLIGONUCLEOTIDES WITH ACTIVITY AGAINST  
HUMAN IMMUNODEFICIENCY VIRUSFIELD OF INVENTION

This invention relates to oligonucleotides particularly useful in inhibiting replication of the human immunodeficiency virus (HIV).

5 BACKGROUND OF THE INVENTION

Antisense oligonucleotides can hybridize with viral mRNA and inhibit translation or processing of mRNA, thereby inhibiting viral replication. Hybridization of antisense oligonucleotides to viral mRNA (antisense:mRNA) occurs by hydrogen bonding between complementary nucleotides present on an antisense oligonucleotide and viral mRNA. Adenine (A) is complementary to thymidine (T) and uracil (U), while cytosine (C) is complementary to guanine (G). Along the antisense:mRNA chain classical base pairs AU, TA or UA, GC, or CG are present. Additionally, some mismatched base pairs (e.g., AG, GU) may be present.

The ability to form hydrogen bonds between nucleotides enables antisense oligonucleotides to be targeted to specific viral nucleic acid sequences. Thus, antisense oligonucleotides can be targeted to nucleic acid sequences present only in viral nucleic acid, and viral gene expression can be selectively inhibited.

Oligonucleotides have been claimed as anti-viral agents able to hybridize with viral nucleic acid. For example, Tullis, U.S. Patent No. 5,023,243 provides a general description of use of antisense oligonucleotides. Kaji, U.S. Patent No. 4,689,320, provides data showing a decrease in mortality in mice infected with Herpes Simplex Virus when treated with an antisense oligonucleotide having a nucleotide sequence targeted to Herpes Simplex Virus. Goodchild et al., U.S. Patent No. 4,806,463,

provide data to demonstrate the ability of several different specified antisense oligonucleotides to inhibit HTLV-III (HIV) replication, and gene expression in cultured cells infected with HIV. Cantin et al., U.S. Patent No. 5,110,802, describe the use of a particular methylphosphonate linked oligonucleotide to inhibit HIV replication. Ecker, U.S. Patent No. 5,166,195, provides data showing the inhibition of a cloned tat gene using certain antisense oligonucleotides (these U.S. patents are hereby incorporated by reference herein). Matsukura et al., Proc. Natl. Acad. 86:4244, describe the inhibition of HIV expression in a chronically infected cell without killing the host cell using a phosphorothioate linked oligonucleotide targeted to a rev sequence.

15 In addition to inhibiting viruses by an antisense mechanism, oligonucleotides can inhibit viruses in a sequence non-specific mechanism. For example, Majumdar et al., Biochemistry 28:1340 (1989) describe the use of phosphorothioate oligonucleotides to inhibit HIV reverse transcriptase.

#### SUMMARY OF THE INVENTION

The present invention describes compounds and methods for inhibiting replication of human immunodeficiency virus (HIV). Preferred HIV target sites are identified and oligonucleotides designed to hybridize to a target site are described. The preferred use of the oligonucleotides is as an anti-HIV agent to inhibit HIV replication in a patient infected with HIV. Other uses of the present invention include detecting the presence of HIV by using the oligonucleotides as detection probes or amplification primers, and measuring the ability of an oligonucleotide to inhibit HIV replication to evaluate its suitability as an anti-HIV agent for a phenotype of HIV or diagnose the presence of HIV in a patient.

35 The described oligonucleotides are targeted to specific HIV nucleic acid sequences and are believed to

inhibit HIV replication by a combination of different mechanisms. One expected mechanism involves hybridization of the oligonucleotide to HIV target nucleic acid thereby inhibiting protein production from the HIV target nucleic acid. Other mechanisms may also play a role in inhibition of HIV replication by the oligonucleotides of this invention. There is no intention to exclude or ignore the possibility that the described oligonucleotides are exerting their effect by mechanisms other than an antisense mechanism. Indeed, phosphorothioate oligonucleotides can inhibit HIV replication by a mechanism not involving oligonucleotide:target hybridization; phosphorothioate oligonucleotides inhibit HIV reverse transcriptase, and may also be able to inhibit gp120 binding and the phosphorylating activity of protein kinase C (PKC).

The described oligonucleotides are targeted to conserved nucleotide sequence regions of the HIV genome coding for proteins necessary for viral replication, namely, mRNA sequences present in the primer binding site (pBS), *psi*, *tat*, *vpr*, *rev*, *env*, and *nef*. Several oligonucleotides targeted to a nucleic acid sequence present in *tat* and *vpr* ("target site 1," SEQ. ID. NO. 94) or to a nucleotide sequence present in *tat*, *rev*, and *env*, as well as *nef* mRNA ("target site 2," SEQ. ID. NO. 95) are particularly effective in inhibiting HIV replication. Preferred oligonucleotides are perfectly complementary to a nucleic acid sequence region present in target site 1 or target site 2. Two examples of these preferred oligonucleotides are given by nucleic acid sequence SEQ. ID. NO. 35, which is perfectly complementary to target site 1, and nucleic acid sequence SEQ. ID. NO. 18, which is perfectly complementary to target site 2.

Thus, in a first aspect, the invention features a method for inhibiting HIV replication using a purified oligonucleotide. The method involves the step of contacting cells infected with HIV with a purified

oligonucleotide 20 to 100 nucleotides in length. The oligonucleotide is substantially complementary to a nucleic acid sequence region of 20 nucleotides present in an HIV nucleic acid sequence of SEQ ID NO: 94 or SEQ ID NO: 95. The oligonucleotide is expected to inhibit HIV replication by reducing the normal activity of HIV nucleic acid needed for replication, thereby inhibiting HIV replication. Normal activity of HIV nucleic acid needed for replication includes reverse transcription of HIV RNA, synthesis of HIV mRNA, translation of HIV mRNA, processing of HIV mRNA and packaging of genomic HIV RNA.

*In vivo* hybridization conditions include a physiological temperature of about 37°C. *In vivo* conditions are low stringency conditions, compared to probe diagnostic hybridization assay conditions, due to their relatively low temperature and hybridization between nucleic acids which are not perfectly complementary can occur. The necessary degree of complementarity for hybridization *in vivo* or *in vitro* will be affected by factors such as the segment length of contiguous complementary bases, the type of bases involved in hydrogen bonding (e.g., G:C hydrogen bond formation is stronger than A:T), internal additions or deletions, and structural chemical modification of the oligonucleotide. Preferably two or less, more preferably one or less, most preferably zero, base pairs in a fifteen base stretch of "contiguous" complementary bases are mismatched, are internally deleted, and/or are internally added, compared to a perfectly complementary nucleic acid strand. Complementarity of a particular oligonucleotide to HIV will depend upon the actual HIV strain or isoforms infecting a patient since the base sequence of each HIV type can differ.

By "hybridize" is meant that the antisense oligonucleotide can form a stable duplex (i.e., one that can be detected) with the target HIV nucleic acid and thereby reduce the normal activity of the nucleic acid.

Hybridization between the oligonucleotide and the target HIV nucleic acid is preferably specific to targeted viral nucleic acid under *in vivo* conditions. However, anti-viral oligonucleotides which can form a duplex with both  
5 the viral target site and non-target cellular sites under *in vivo* conditions may still be useful as anti-viral agents *in vivo*. *In vivo* effectiveness may be determined by different factors, such as adequate discrimination between inhibition of viral nucleic acid activity and  
10 cellular nucleic acid activity, or through an effect on a cellular factor required for HIV expression.

"Substantially complementary to" a nucleic acid sequence means the oligonucleotide is capable of hybridizing to the nucleic acid sequence to form a  
15 detectable duplex and preferably has a 0 to 10%, preferably 0 to 5% nucleotide base difference (excluding RNA or DNA equivalent nucleotides), from a nucleic acid perfectly complementary to the nucleic acid sequence. Nucleotide base differences include mismatches, internal  
20 additions and/or internal deletions.

The term "purified" refers to an oligonucleotide in a form not found in nature without human intervention. Such oligonucleotides include oligonucleotides isolated, to some extent, and recombined with foreign nucleic acid.  
25 Purified oligonucleotides may be produced by techniques known in the art such as chemical synthesis, and *in vitro* or *in vivo* expression from recombinant nucleic acid molecules, e.g., retroviral vectors.

Other aspects describe methods of inhibiting HIV  
30 replication using a purified oligonucleotide consisting essentially, having, or substantially complementary, to a nucleotide sequence region selected from the group consisting of SEQ. ID. NOs. 18-20 and 22-29, and the RNA equivalents thereto by SEQ. ID. NOs. 65-67 and 69-76  
35 (target site 2); and SEQ. ID. NOs. 35, 38-46, and the RNA equivalents thereto by SEQ. ID. NOs. 82, 85-93 (target site 1).

"Consisting essentially" of a nucleic acid sequence means the oligonucleotide contains a nucleic acid sequence which has 0 to 10%, preferably 0 to 5% nucleotide base difference (excluding RNA or DNA equivalent nucleotides),  
5 from the specified nucleotide sequence and has the claimed activity (e.g., anti-HIV activity). Nucleotide base differences include mismatches, internal additions and/or internal deletions. In addition the phrase "consisting essentially" also provides a size limitation of up to 4  
10 additional nucleotides or up to two outside deletions. The additional nucleotides may be complementary, or non-complementary, to HIV nucleic acid.

"RNA and DNA equivalents" refer to RNA and DNA molecules having the same complementary base pair  
15 hybridization properties. RNA and DNA equivalents have different sugar groups (i.e., ribose versus deoxyribose), and may differ by the presence of uracil in RNA and thymine in DNA. RNA and DNA equivalents have the same degree of complementarity to a particular sequence.

20 Preferably, the oligonucleotide is used in the therapeutic treatment of a patient infected with HIV. "Therapeutic" refers to the treatment or prevention of disease caused by HIV infection. A "therapeutically effective amount" is one which inhibits replication of HIV  
25 in a patient infected with HIV. Preferably, the therapeutically effective amount relieves, to some extent, one or more symptom associated with HIV infection.

Another aspect describes oligonucleotides targeted to HIV nucleic acid comprising a purified oligonucleotide  
30 to 100 nucleotides substantially complementary to a nucleic acid sequence region of 20 nucleotides present in target site 1 or target site 2.

In other aspects, the invention features oligonucleotides having, consisting essentially of, or  
35 substantially corresponding to specified nucleic acid sequences targeted (i.e., complementary) to HIV nucleic acid sequences present in target site 1 or target site 2.



The specific nucleic acid sequences of oligonucleotides to target site 2 are given by SEQ. ID. NOs. 18-20 and 22-29, and the RNA equivalents thereto by SEQ. ID. NOs. 65-67 and 69-76. The specific nucleic acid sequences of oligonucleotides to target site 1 are given by SEQ. ID. NOs. 35, 38-46, and the RNA equivalents thereto by SEQ. ID. NOs. 82, 85-93.

The oligonucleotides of this invention are generally synthesized *in vitro* and can be introduced into a cell as a therapeutic composition. The oligonucleotides can also be introduced into a cell by a vector containing the nucleic acid sequences of the target site. Such a vector encodes RNA which can function as an antisense oligonucleotide. See Izant et al., Science 229:345-352 (1985).

The nucleotide subunits of an individual oligonucleotide may be joined by phosphodiester linkages or modified linkages or by non-nucleotide moieties which do not prevent the hybridization properties of the antisense molecule. Modified linkages include those linkages in which a standard phosphodiester linkage is replaced with a different linkage, such as a phosphorothioate, methylphosphonate, phosphodithioate, or phosphoselenate linkage. Modified linkages are generally more resistant to nuclease degradation than phosphodiester linkages.

Oligonucleotide modifications which do not prevent the oligonucleotide from hybridizing to its target nucleic acid are also covered by the present invention. Modified oligonucleotides can have modified sugar groups which do not prevent the oligonucleotide from inhibiting HIV activity or replication. Similarly, oligonucleotides can have modified purine or pyrimidine bases which do not prevent the oligonucleotide from inhibiting HIV.

Oligonucleotides containing phosphorothioate linkages targeted to target sites 1 and 2 were found to inhibit HIV gene expression. Indeed, antisense oligonucleotides

targeted to target sites 1 and 2 containing from 13 - 100 nucleotides covalently joined together are active in this invention.

The invention also features methods for use of the  
5 above oligonucleotides, and vectors encoding such oligonucleotides. Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

10 DETAILED DESCRIPTION OF THE INVENTION

The present invention concerns oligonucleotides targeted to the HIV genome and mRNA's. The oligonucleotides are designed to hybridize to a particular HIV nucleic acid sequence and are preferably used to  
15 inhibit HIV replication. The oligonucleotides have various uses relating to their ability to inhibit HIV replication and/or hybridize to a HIV nucleic acid sequence. Such uses include use as a therapeutic agent and use in diagnostic assays.

20 The anti-HIV oligonucleotides can be used to inhibit HIV replication alone or in combination with other anti-HIV oligonucleotide or anti-HIV treatments. For example, a first anti-HIV oligonucleotide can be used in combination with a second anti-HIV oligonucleotide. The  
25 second anti-HIV oligonucleotide may be either, 1) a subtargeted oligonucleotide, 2) a second separately targeted oligonucleotide, or 3) a non-targeted phosphorothioate oligonucleotide. A subtargeted oligonucleotide is designed to hybridize to a subtarget  
30 region of the target region of the first targeted oligonucleotide. Thus, a subtargeted oligonucleotide contains a truncated portion of a first targeted oligonucleotide. An example of a subtargeted/targeted oligonucleotide combination is the phosphorothioate  
35 oligonucleotide of sequence SEQ. ID. NO:18 and the

phosphorothioate oligonucleotide of sequence SEQ. ID. NO:22, in a 10 to 1 ratio.

The following nucleic acid sequences are provided:

TABLE 1

|    |                  |            |             |           |
|----|------------------|------------|-------------|-----------|
| 5  | SEQ. ID. NO. 1:  | CTTTCAGGTC | CCTGTTCGGG  | CGCCACT,  |
|    | SEQ. ID. NO. 2:  | CTTTCAGGTC | CCTGTTCGGG  | CGCCACT,  |
|    | SEQ. ID. NO. 3:  | CTTCTAGCCT | CCGCTAGTCA  | AAATTTT,  |
|    | SEQ. ID. NO. 4:  | CTTCTAGCCT | CCGCTAGTCA  | AAAATATT, |
|    | SEQ. ID. NO. 5:  | CTTCTAGCCT | CCGCTAGTCA  | AAATT,    |
| 10 | SEQ. ID. NO. 6:  | CTCCTTCTAG | CCTCCGCTAG  | TCAAAAAT, |
|    | SEQ. ID. NO. 7:  | CTCTCTCCTT | CTAGCCTCCG  | CTAGTCAA, |
|    | SEQ. ID. NO. 8:  | TTAATACTGA | CGCTCTCGCA  | CCCATCT,  |
|    | SEQ. ID. NO. 9:  | ACAACAGACG | GGCACACACT  | AACT,     |
|    | SEQ. ID. NO. 10: | GAGAGAGCTC | TGGTTTCCCT  | TT,       |
| 15 | SEQ. ID. NO. 11: | TCGCCGCCCC | TCGCCTCTTG  | CCGTGC,   |
|    | SEQ. ID. NO. 12: | TCCTGCCATA | GGAGATGCCT  | AAGGCC,   |
|    | SEQ. ID. NO. 13: | TTGATGAGTC | TGACTGTTCT  | GAT,      |
|    | SEQ. ID. NO. 14: | TTGGGAGGTG | GGTTGCTTTG, |           |
|    | SEQ. ID. NO. 15: | TCTCTCTCTC | CACCTTCTTC  | TTCTAT,   |
| 20 | SEQ. ID. NO. 16: | TAATCGAATG | GATCTGTCTC  | TGTCTC,   |
|    | SEQ. ID. NO. 17: | GAGCTCTTCG | TCGCTGTCTC  | CGCTTCT,  |
|    | SEQ. ID. NO. 18: | CTTCGGGCCT | GTCGGGTCCC  | CTCGGG,   |
|    | SEQ. ID. NO. 19: | GCCTGTCGGG | TCC,        |           |
|    | SEQ. ID. NO. 20: | GCCTGTCGGG | TCCT,       |           |
| 25 | SEQ. ID. NO. 21: | CGGGTCCCCT | CGGG,       |           |
|    | SEQ. ID. NO. 22: | GCCTGTCGGG | TCCC,       |           |
|    | SEQ. ID. NO. 23: | CTTCGGGCCT | GTCG,       |           |
|    | SEQ. ID. NO. 24: | CTTCGGGCCT | GTCGGGTC,   |           |
|    | SEQ. ID. NO. 25: | GGGCCTGTCG | GGTCCCCT,   |           |
| 30 | SEQ. ID. NO. 26: | CTGTCGGGTC | CCCTCGGG,   |           |
|    | SEQ. ID. NO. 27: | TCGGGCCTGT | CGGGTCCCCT  | CG,       |
|    | SEQ. ID. NO. 28: | CTTCGGGCCT | GTCGGGTCCC  | CT,       |
|    | SEQ. ID. NO. 29: | GGGCCTGTCG | GGTCCCCTCG  | GG,       |
|    | SEQ. ID. NO. 30: | TAGGATCTAC | TGGCTCCATT  | TC,       |
| 35 | SEQ. ID. NO. 31: | ATTGGTACAA | GCAGTTTTAG  | GCT,      |
|    | SEQ. ID. NO. 32: | AGTGGTACAA | GCAGTTTTAG  | GCT,      |
|    | SEQ. ID. NO. 33: | GCTTCTTCCT | GCCATAGGAG  | A,        |

SEQ. ID. NO. 34: GACTGTTCTG ATGAGCTCTT CGTC,  
SEQ. ID. NO. 35: GCCTATTCTG CTATGTCGAC ACCCAA,  
SEQ. ID. NO. 36: CGGGCCTGTC GGGTCCCCTC GGGA,  
SEQ. ID. NO. 37: CGGGCCTGTC GGGTCCCCTC GGGG,  
5 SEQ. ID. NO. 38: TCTGCTATGT CGAC,  
SEQ. ID. NO. 39: ATGTCGACAC CCAA,  
SEQ. ID. NO. 40: GCCTATTCTG CTAT,  
SEQ. ID. NO. 41: ATTCTGCTAT GTCGACAC,  
SEQ. ID. NO. 42: TGCTATGTCG ACACCCAA,  
10 SEQ. ID. NO. 43: GCCTATTCTG CTATGTCG,  
SEQ. ID. NO. 44: GCCTATTCTG CTATGTCGAC AC,  
SEQ. ID. NO. 45: CTATTCTGCT ATGTCGACAC CC,  
SEQ. ID. NO. 46: ATTCTGCTAT GTCGACACCC AA,

Oligonucleotides containing these sequences, consisting of  
15 these sequence, containing sequences substantially  
corresponding to these sequences, consisting essentially  
of these sequences, are useful in one or more aspects of  
the present invention.

"Substantially corresponding" refers to an  
20 oligonucleotide having a nucleic acid sequence which is  
identical to, or has no more than a 20% nucleotide base  
difference (excluding RNA or DNA equivalent nucleotides),  
from a specified sequence and has the claimed activity  
(e.g., anti-HIV activity). Nucleotide base differences  
25 include mismatches, internal additions and/or internal  
deletions. Additional nucleotides outside of the specified  
sequence may be present. The additional nucleotides may  
be complementary or non-complementary to HIV nucleic acid.  
Preferably, the substantially corresponding sequence  
30 differs by no more than 10%, more preferably no more than  
5% from the specified sequence.

#### I. INHIBITION OF HIV PROPAGATION

Oligonucleotides targeted to HIV target sites 1 and 2,  
are particularly effective in inhibiting HIV gene  
35 expression. Target site 1 is present in exons coding for  
tat and vpr. Target site 2 is present in exons coding for

tat, rev, env, and nef. The presence of the same target site in more than one gene is due to overlapping exons in the HIV genome. As a result of the overlapping exons several genes in the HIV genome contain many of the same nucleic acid sequences in one or more reading frames. Schwartz et al., Journal of Virology 64:2519 (1990).

The design of antisense oligonucleotides targeted to HIV is described in detail below. The examples that follow are not limiting in the invention; those skilled in the art will recognize that equivalent oligonucleotides targeted to the regions described below can be readily designed and synthesized, using this application as a guide.

#### A. Selection Of Target Sites 1 And 2

Antisense oligonucleotides were targeted to conserved HIV nucleic acid sequences having either a weak or a strong secondary structure. The primary sequence of different strains of HIV was obtained from various publications describing the HIV nucleic acid sequence. Antisense oligonucleotides were designed based upon the HIV nucleic acid sequence published for HXB-2 strain of HIV. Conserved primary nucleic acid sequences coding for essential viral proteins were selected by comparing the published HIV sequences for different strains of HIV. The secondary structure was predicted from the primary nucleic acid sequence using methods known in the art (e.g., Zucker, M., In Methods In Enzymology, vol 190, p. 262 (1979)).

Antisense oligonucleotides were targeted to nucleic acid sequences having either a weak or a strong secondary structure, based upon different rationales. A secondary structure arises from the formation of intramolecular hydrogen bonds between complementary nucleotides. The weaker the secondary structure the less likely the formation of intramolecular hydrogen bonds.

B. Design of Antisense Oligonucleotides Targeted  
To Target Sites 1 and 2

The nucleotide sequences of target sites 1 and 2 are indicated by SEQ. ID. NOs. 94 and 95 respectively. The ability of oligonucleotides having nucleic acid sequences complementary to these sequences to inhibit HIV replication indicate that other antisense oligonucleotides targeted to SEQ. ID. NOs. 94 or 95 will be effective in inhibiting HIV gene expression. SEQ. ID. NOs. 18 and 35, are complementary to SEQ. ID. NOs. 95 and 94, respectively. Both longer versions and truncated versions of SEQ. ID. NOs. 18 and 35 can be used to inhibit HIV replication.

Several factors are important in determining the appropriate versions of SEQ. ID. NOs. 18 and 35 used to inhibit HIV replication. Versions of SEQ. ID. NOs. 18 and 35 must be able to hybridize sufficiently with SEQ. ID. NOs. 95 or 94 under physiological conditions to inhibit nucleic acid activity. Factors affecting hybridization include, base composition, oligonucleotide modifications and oligonucleotide size.

Other factors which are important in oligonucleotide design are the length of the complementary region, the percentage of guanine and cytosine residues, the type of oligonucleotide linkages, and accessibility of the target sequence to hybridization. These factors are known in the art. See, Britten, R.J. and Davidson, E. H., In Hybridization Strategy In Nucleic Acid Hybridization, (eds. B. D. Hames and S.J. Higgins) IRL Press, Washington D.C. (1985), hereby incorporated by reference herein. Anti-HIV oligonucleotides are preferably 18 to 50 nucleotides in length, more preferably, 20-35 nucleotides in length. The importance of these factors in a given case can be initially determined *in vitro*, followed by *in vivo* studies.

### 1. Oligonucleotide Modification

Oligonucleotides can be modified to enhance their anti-HIV activity and therapeutic efficacy. Preferred modifications enhance oligonucleotide cellular uptake, oligonucleotide stability, and inhibit HIV propagation. Modified oligonucleotides having increased stability and/or cellular uptake include oligonucleotides having a modified internucleotide linkage, and oligonucleotide modified sugar groups. Examples of modified internucleotide linkages include phosphorothioates, methylphosphonates, and phosphorodithioates. Examples of modified sugar groups include  $\alpha$ -anomers and 2'-O-methyloligonucleotides. Cantin and Woolf Trends in Microbiology 1:270--276, 1993).

Anti-HIV oligonucleotides preferably contain phosphorothioate linkages. Phosphorothioate linkages increase oligonucleotide stability, facilitate cellular uptake of oligonucleotides, and enable the oligonucleotide to inhibit HIV propagation by a mechanism which appears to be partly sequence independent. Thus, phosphorothioate linked oligonucleotides inhibit HIV by targeting, based on their nucleic acid sequence, a specific HIV target site, and inhibit HIV by a mechanism not dependent on a specific sequence.

Oligonucleotides having phosphorothioate linkages can inhibit viral reverse transcriptase, and may also inhibit gp120 binding to the CD4 receptor and phosphorylating activity of PKC. The viral reverse transcriptase inhibitory effect increases as the size of the phosphorothioate oligonucleotide increases. Oligonucleotides having phosphorothioate linkages are described by Cohen et al., U.S. Patent 5,264,423, and Kinchington et al., Antiviral Research, 17:53-62, 1992.

The cell association of phosphorothioate oligonucleotides, in experiments measuring oligonucleotide uptake and stability, was consistently ten times more than for phosphodiester oligonucleotides. Part of this effect

appears to be due to cellular uptake of phosphorothioate oligonucleotides being greater than that of phosphodiester oligonucleotides, and part of the effect is attributed to increased phosphorothioate oligonucleotide stability. The difference in uptake mechanisms appears have a greater effect on cell association than the differences in oligonucleotide stability.

Thierry and Dritschilo, Nucleic Acids Research 20:5691-5698 (1992), also observed an increase in oligonucleotide uptake and stability due to the presence of phosphorothioate linkages. Thierry found a first oligonucleotide, having 100% phosphorothioate linkages, and a second oligonucleotide, end capped with phosphorothioate groups (*i.e.*, an oligonucleotide having two phosphorothioate modifications at both ends), were both more stable and taken up by a cell to a greater extent than an oligonucleotide having only phosphodiester linkages.

## 2. Oligonucleotide Size

The optimal oligonucleotide size should take into account several factors including different anti-HIV mechanisms and cellular uptake. Anti-HIV oligonucleotides are preferably 18 to 100 nucleotides in length and contain a preferred nucleic acid sequence or a nucleic acid sequence substantially corresponding to a preferred nucleic acid sequence. Such oligonucleotide are targeted to the identified target site. Additional nucleotides of the preferred nucleic acid sequence may be complementary to HIV nucleic acid or may be non-complementary. More preferably, these oligonucleotides are 18 to 50 nucleotides in length. Most preferably, the oligonucleotide are 20-35 nucleotides in length.

Possible disadvantages of oligonucleotides having a longer length include a decrease in oligonucleotide uptake and a possible increase in cytotoxic effect. The degree of these effects are, at least in part, determined by the



size of the oligonucleotide and types of oligonucleotide linkages. Possible cytotoxic effects may be more pronounced, for example, using oligonucleotides containing phosphorothioate linkages.

5 Oligonucleotides containing longer nucleic acid sequences having complementarity to a target sequence offer several advantages compared to shorter oligonucleotides, including increased target specificity and increased stability of the oligonucleotide:target  
10 duplex. The increased stability of the oligonucleotide:target duplex may facilitate the oligonucleotide's HIV inhibitory effect in different ways. For example, if the primary effect is translation arrest, the increased stability of the duplex could increase  
15 translation arrest by preventing a ribosome from displacing the oligonucleotide.

Another example of a possible mechanism involves degradation of the RNA strand of an DNA:RNA HIV duplex with an enzyme having RNase H activity. In this instance,  
20 the increased stability of the duplex increases the likelihood that the duplex is acted on by the enzyme. To be degraded by RNase H activity, the anti-HIV oligonucleotide in the DNA:RNA HIV duplex preferably contains three or more contiguous phosphodiester or  
25 phosphorothioate linkages.

### 3. Oligonucleotide Complementarity and Melting Temperature

As discussed above, complementary oligonucleotides are designed to hybridize to a target sequence region. While  
30 nucleic acids that are not perfectly complementary may hybridize to each other under physiological conditions, the longest stretch of perfectly complementary base sequence generally determines hybrid stability. Oligonucleotides designed to hybridize to a particular  
35 sequence region should be designed to have an appropriate

melting temperature ( $T_m$ ) (the temperature at which 50% of the oligonucleotide is hybridized to its target nucleic acid). The appropriate  $T_m$  can be obtained by varying the probe length and nucleotide composition (percentage of G + C versus A + T). The probe length and nucleotide composition should result in a  $T_m$  about 2-10 °C higher than physiological temperature (37°C).

The longer the complementary region on an oligonucleotide, the more hydrogen bonding to a target sequence, and in general, the higher the  $T_m$ . Increasing the percentage of G and C also increases the  $T_m$  because G-C base pairs exhibit stronger hydrogen bonding and therefore greater thermal stability than A-T base pairs.  $T_m$  can be determined using techniques known in the art such as measuring hybridization by the hybridization protection assay (HPA) according to Arnold et al., entitled "Homogeneous Protection Assay," EPO application number 88308767.8, publication number 309230, and Nelson et al., in Nonisotopic DNA Probe Techniques, p. 275 Academic Press, San Diego (Kricka, ed., 1992) (these references are hereby incorporated by reference herein). Oligonucleotides can be labeled with acridinium ester derivatives as described by Arnold, et al., PCT/US88/03361, entitled "Acridinium Ester Labeling and Purification of Nucleotide Probes," hereby incorporated by reference herein.

$T_m$  can be measured using HPA in the following manner. Oligonucleotides are labeled with an acridinium ester. Oligonucleotide:target hybrids are formed in a lithium succinate buffer (0.1 M lithium succinate buffer (pH 5.0), 2 mM ethylenediaminetetraacetic acid (EDTA), 2 mM ethylene glycol bis (beta-amino ethyl ether) N, N, N', N' tetraacetic acid (EGTA), 10% (w/v) lithium lauryl sulfate) using an excess amount of HIV RNA target. Aliquots of the solution containing the nucleic acid hybrids are then diluted in the lithium succinate buffer solution. The aliquots are incubated for five minutes at various

temperatures starting below that of the anticipated  $T_m$  and increasing in 2-5°C increments. This solution is then diluted with a mild alkaline borate buffer (0.15 M sodium tetraborate, (pH 7.6), 5% (v/v) TRITON® X-100) and  
5 incubated at a lower temperature for ten minutes. Under these conditions the acridinium esters attached to single-stranded oligonucleotides are hydrolyzed, while acridinium esters attached to hybridized oligonucleotides are relatively protected from hydrolysis. Thus, the amount of  
10 acridinium esters remaining after hydrolysis treatment is proportional to the number of hybrid molecules present in the sample. The remaining acridinium esters can be measured by monitoring the chemiluminescence produced by addition of hydrogen peroxide and alkali to the solution.  
15 Chemiluminescence can be measured in a luminometer (e.g., the Gen-Probe LEADER® I or LEADER® 50). The resulting data is plotted as percent of maximum signal (usually from the lowest temperature) versus temperature. In this assay, the  $T_m$  is determined to be the temperature at which 50% of  
20 the maximum signal remains. In addition to the method above,  $T_m$  may be determined by isotopic methods well known to those skilled in the art (see e.g., Hogan et al., supra).

#### 4. Screening Assay

25 The oligonucleotide can also be screened by an oligonucleotide screening assay designed to mimic physiological conditions to obtain a measure of the hybridization expected to occur under physiological conditions. Due to the complexity of physiological  
30 conditions, the oligonucleotide screening assay provides an approximation rather than an exact prediction of actual hybridization behavior in a cell. An oligonucleotide screening assay can be carried out using a test DNA oligonucleotide, an acridinium ester labeled  
35 oligonucleotide having the same nucleotide sequence as the

test oligonucleotide, and an enzyme having RNase H activity.

The assay measures the ability of the DNA oligonucleotide to hybridize to an RNA target, thereby forming an DNA:RNA duplex, by measuring the subsequent degradation of the target RNA by RNase H activity. The acridinium ester labeled oligonucleotide is used to detect remaining target nucleic acids.

An oligonucleotide screening assay can be carried out as follows:

- 1) Hybridize oligonucleotides to their target nucleic acids in a solution, such as an aqueous physiological buffer. An example of a target nucleic acid is purified HIV mRNA. Hybridization can be carried out using 0.9 pmol of target mRNA, 0.1 pmol acridinium ester-labeled probe, in 100  $\mu$ L of a physiological buffer, at 37°C for 2 hours. The reactions are divided to make duplicates at 1 x final buffer concentration for optimal RNase H enzyme activity.
- 2) E. coli RNase H (Life Technologies, Gaithersburg, MD, 0.4 U/reaction) is added to one of the two duplicate reactions. The other duplicate reaction lacks RNase H and serves as the (-) RNase H control. The reactions are incubated at 37°C for 1 hour, stopped by denaturing at 95°C for 5 minutes, and placed directly on ice.
- 3) Aliquots of the reactions are hybridized with the appropriate phosphodiester acridinium ester-probe. Appropriate acridinium ester-labeled probes can hybridize to the same nucleic acid sequence as the test oligonucleotide and contain an acridinium ester in the complementary region. The acridinium ester-probe is

hybridized at 60°C for 1 hour. Control hybridizations are performed using acridinium ester-probes expected to hybridize to a region other than the target nucleic acid sequence.

5

- 4) Aliquots are diluted in hybridization buffer (0.1 M lithium succinate buffer (pH 5.0), 2 mM EDTA, 2 mM EGTA, 10% (w/v) lithium lauryl sulfate). Fifty microliter replicates are hydrolyzed in 12 x 75 mm luminometer tubes with 300  $\mu$ L of 0.15 M sodium tetraborate (pH 7.6), 5% (v/v) TRITON® X-100 at 60°C until non-hybridized labeled probes are fully hydrolyzed (usually 6-8 minutes). Chemiluminescence is brought about using a single injection of 1.5 N NaOH, 0.1% H<sub>2</sub>O<sub>2</sub> and measured in a luminometer.

10

15

As would be appreciated by one skilled in the art, variations of this procedure can be performed. For example, the assay can be carried out using different amounts of reagents and incubation times.

20

### C. Therapeutic Activity

The ability of oligonucleotides described herein to inhibit HIV replication is not limited to any particular theory. It is believed that the described oligonucleotides can inhibit viral replication by hybridizing sufficiently in vivo to viral nucleic acid to inhibit viral nucleic acid activity.

25

#### 1. Discrimination of Target

The described oligonucleotides can function as therapeutic agents by hybridizing sufficiently to viral nucleic acid to inhibit viral nucleic acid activity while not hybridizing sufficiently to essential cellular nucleic acid to inhibit essential cellular nucleic acid activity. Essential cellular nucleic acid activity is nucleic

30

35

activity needed for cell growth or sustenance. Nucleic acid activity of cellular nucleic acid includes translation and processing of cellular mRNA.

Antisense oligonucleotides which cannot adequately discriminate between viral and essential cellular nucleic acid may be able to act as a therapeutic by being delivered only to HIV infected cells (e.g., using liposomes containing recognition molecules targeted to HIV infected cells).

An estimate of the relative potential activity of different oligonucleotides can be assessed by measuring the hybridization behavior of the oligonucleotide. Exact duplication of *in vivo* conditions is extremely difficult because the intracellular environment is very complicated and not completely known. Furthermore, there are many nucleic acid binding proteins present in cells which may effect accessibility and activity of viral targets.

*In vivo* hybridization activity can be modeled *in vitro* (e.g., using standard physiological saline or 0.12 M phosphate buffer pH ~7.0 at 37°C). Hybridization can be determined using an *in vitro* quantitative assay, according to Kohne U.S. Patent No. 4,851,330, or Maniatis et al. Molecular Cloning, Cold Spring Harbor Press, N.Y. (1982), both references are hereby incorporated by reference herein. Preferably, antisense oligonucleotides specifically hybridize to target mRNA *in vivo*. However, absolute specificity is not required. Rather, the antisense oligonucleotide should be able to adequately discriminate between viral and cellular nucleic acid, such that viral propagation can be inhibited without causing significant cell toxicity. Significant cell toxicity occurs when cell growth is inhibited to a greater extent than viral propagation at a particular oligonucleotide concentration. Cell toxicity and compound efficacy can be determined using different techniques. Initial screening can be performed by characterizing the oligonucleotide's ability to hybridize to a target sequence. Subsequent

toxicity and/or efficacy tests can be performed using cell cultures and animal models.

The ability to discriminate between cellular and viral nucleic acid activity *in vivo* can be estimated by measuring specificity of an antisense oligonucleotide *in vitro*. The measured ratio of efficacy/toxicity for antisense mechanisms is expected to increase with increasing specificity. Specificity can be measured under stringent hybridization conditions optimized for this purpose using total cellular nucleic acid. Preferably, acridinium ester (AE) labelled nucleotides are used to determine specificity, and hybridization is detected using the Hybridization Protection Assay (HPA) according to Arnold et al., entitled "Homogeneous Protection Assay," EPO application number 88308767.8, publication number 309230, hereby incorporated by reference herein.

Examples of stringent hybridization conditions for use with AE labelled oligonucleotides include: (1) hybridization at 50°C for one hour in a solution containing 0.095 M lithium succinate pH 5, 0.31 M lithium lauryl sulfate, 1.5 mM EDTA, 1.5 mM EGTA; and (2) 0.05 M lithium succinate pH 5, 0.6 M LiCl, 1% (w/v) lithium lauryl sulfate, 10 mM EDTA, 10 mM EGTA at 50°C for 15 minutes, followed by the addition of 300 µl of 0.6 M sodium borate pH 8.5, 1% TRITON® X-100 at 60°C for 5-7 minutes.

Hybridization can be judged specific for HIV if the antisense oligonucleotide hybridizes to nucleic acid (total cellular RNA and/or ssDNA) isolated from cells infected with HIV, two times greater than the background signal obtained from the control experiment. The control experiment contains only cellular nucleic acid. Preferably, the signal resulting from hybridization to HIV nucleic acid is at least five times greater than background signal.

## 2. Administration of Oligonucleotides

The described oligonucleotides are useful for treating patients infected with HIV by inhibiting HIV nucleic acid activity. Anti-viral oligonucleotides can be used to  
5 treat HIV patients using different formulations and routes of administration. Suitable routes of administration include intramuscular, aerosol, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and intrathecal.

10 Antisense oligonucleotides may be introduced as naked oligonucleotide, through expression vectors (encoding such antisense molecules), or as physiologically acceptable formulations. Suitable formulations include the use of a liposome, a controlled release vehicle, by use of  
15 iontophoresis, ion paired molecules, covalently attached adducts, and other pharmacologically suitable methods of delivery.

Different types of delivery strategies are useful in the present invention, including: oligonucleotide  
20 modifications, particle carrier drug delivery vehicles, and retroviral expression vectors. Antisense oligonucleotides joined by phosphodiester linkages are slowly taken up by cells. To enhance cellular uptake, the antisense oligonucleotide may be modified at the  
25 phosphodiester linkage to reduce its charge. For example, the individual nucleotides may be joined by methylphosphonate linkages.

Modification of antisense to reduce charge is just one approach to enhance the cellular uptake of these  
30 larger molecules. Modifications can also be designed to reduce susceptibility to nuclease degradation (e.g., use of phosphorothioate linkages).

Drug delivery vehicles can be chosen for both systemic and topical administration. These vehicles can  
35 be designed to serve as a slow release reservoir or to deliver their contents directly to the target cell. An advantage of using direct delivery drug vehicles is that



multiple molecules are delivered per uptake. Such vehicles have been shown to increase the circulation half-life of drugs which would otherwise be rapidly cleared from the blood stream. Some examples of such specialized drug delivery vehicles which fall into this category are liposomes, hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres.

The use of liposomes as a drug delivery offers several advantages. Liposomes increase intracellular stability, increase uptake efficiency and improve biological activity. Liposomes are hollow spherical vesicles composed of lipids arranged in a similar fashion as those lipids which make up the cell membrane. They have an internal aqueous space for entrapping water soluble compounds and range in size from 0.05 to several microns in diameter. Several studies have shown that liposomes can deliver nucleic acids to cells and that the nucleic acid remains biologically active. For example, a liposome delivery vehicle originally designed as a research tool, Lipofectin, can deliver intact nucleic acid molecules to cells.

Specific advantages of using liposomes include the following: they are non-toxic and biodegradable in composition; they display long circulation half-lives; and recognition molecules can be readily attached to their surface for targeting to tissues. Finally, cost effective manufacture of a liposome-based pharmaceutical, either in a liquid suspension or lyophilized product, has demonstrated the viability of this technology as an acceptable drug delivery system.

Antisense oligonucleotides may be systemically administered. Systemic absorption refers to the accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes which lead to systemic absorption include: intravenous, subcutaneous, intraperitoneal, intranasal, intrathecal and ophthalmic. Each of these administration

routes expose the antisense oligonucleotide to accessible diseased cells. Subcutaneous administration drains into a localized lymph node which proceeds through the lymphatic network into the circulation. The rate of entry  
5 into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier can localize the antisense oligonucleotide at the lymph node. The antisense oligonucleotide can be modified to diffuse into the cell, or the liposome can  
10 directly participate in the delivery of either the unmodified or modified antisense oligonucleotide to the cell.

A liposome formulation which can associate antisense oligonucleotides with the surface of lymphocytes and  
15 macrophages is also useful. This will provide enhanced delivery to HIV-infected cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of infected cells.

Intraperitoneal administration also leads to entry  
20 into the circulation with the molecular weight or size of the antisense oligonucleotide-delivery vehicle complex controlling the rate of entry.

The chosen method of delivery will result in intracellular accumulation of the composition in the  
25 afflicted cells. Nuclear delivery may be used but is less preferable. The accumulation of oligonucleotide in a particular compartment will be largely effected by oligonucleotide charge and chemistry. Most preferred delivery methods include liposomes (10-400 nm), hydrogels,  
30 controlled-release polymers, microinjection or electroporation (for ex vivo treatments) and other pharmaceutically applicable vehicles.

The exact dosage and number of doses will depend upon the efficacy data from clinical trials. Several factors  
35 such as the delivery vehicle, disease indication, the route of administration, and the linkage joining the oligonucleotide will effect the dosage. The expected

dosage is between 0.001-200 mg/kg of body weight/day. The duration of treatment will extend through the course of the disease symptoms. Multiple daily doses are anticipated for topical applications, ocular applications  
5 and vaginal applications.

Establishment of therapeutic levels of antisense oligonucleotides within the cell is dependent upon the rate of uptake and degradation. Decreasing the degree of degradation prolongs the intracellular half-life of the  
10 antisense oligonucleotide. Thus, chemically modified antisense oligonucleotides, e.g., with modification of the phosphate backbone, are preferred.

### 3. HIV Protection Assay

Anti-HIV oligonucleotides can also be used in assays  
15 measuring the ability of the oligonucleotide to inhibit HIV cytopathic effects. These assays have various uses including use to identify or confirm the presence of HIV as a disease causing agent in a person, use to determine which oligonucleotide to administer to a patient, and use  
20 to evaluate the initial effectiveness of an oligonucleotide.

An HIV protection assay can be carried using anti-HIV oligonucleotides and standard techniques for measuring cell growth. Techniques which can be used as a measure of  
25 cell growth include the use of dyes such as XTT (2,3-bis [2-methoxy-4-nitro-5-sulphophenyl]-2H-tetrazolium-5-carboxanilide) to measure the cells metabolic state, the use of radioactive or modified nucleotide precursors such as BUdR (bromodeoxyuradine) to measure nucleic acid  
30 replication, and the use of oligonucleotides complementary to host nucleic acids to measure production of host nucleic acids.

Assays involving oligonucleotides complementary to cellular nucleic acids can be carried out using an  
35 oligonucleotide containing a detectable label such as fluorescent, chemiluminescent, enzyme or radioactive

label. Oligonucleotides can be designed to hybridize to host nucleic acid sequence regions such as those present in DNA, mRNA or rRNA. Examples of such nucleotide sequence regions are known in the art and can be obtained  
5 by standard techniques. The preferred source of host target nucleic acids to test for growth of host cells is rRNA. A nucleic acid having a nucleotide sequence characteristic of rRNA is generally present in a cell in much greater abundance than a nucleic acid sequence  
10 present in mRNA.

HIV infects cells containing a CD4 antigen (CD4<sup>+</sup>). The major target cell population is T-helper lymphocytes and cells of the monocyte/macrophage lineage. The HIV-protection assay can be performed on such cells from  
15 persons suspected of being infected with HIV. The assay can be performed *in vitro* directly on such cells taken from the patient, or can be performed using lysates obtained from CD4<sup>+</sup> cells. The lysate can be used to infect cells more susceptible to the HIV cytopathic effect than  
20 the isolated cells.

An HIV protection assay can be carried out as follows:

- 1) Isolate CD4<sup>+</sup> cells from a person. Preferred cells are T-lymphocytes.
- 25 2) Incubate cells under conditions compatible with cell growth in the presence (treated cells) and absence (control cells) of an anti-HIV oligonucleotide. Examples of conditions compatible with cell growth are described by S.  
30 Gartner and M. Popovic, 1990, Virus Isolation and Production, pp. 53-70 in Techniques in HIV Research, ed. by A. Alaldocini and B.D. Walker. Stockton Press. N.Y.
- 35 3) Measure the growth of the treated and control cells at one or more time point after exposure of cells to the oligonucleotides.

Normal growth of control cells indicates the absence of a viral infection such as an HIV infection. Normal growth can be determined by comparing the growth of the control cells to the same type of cells which are known to be healthy.

Less than normal growth of control cells indicates the presence of some cellular disorder, such as HIV. The ability of an anti-HIV oligonucleotide to protect against HIV cytotoxicity in treated cells indicates the disorder is due to HIV and that the tested anti-HIV oligonucleotide can be used to treat the patient. The inability of an anti-HIV oligonucleotide to inhibit HIV in this assay cell toxicity may at times fail to correctly indicate the presence of HIV due to the variability in nucleic acid sequences of different strains of HIV. Thus, an HIV protection assay should be used along with other assays known in the art to detect the presence of HIV. Because of the severity of AIDS, and the possible adverse effect an incorrect diagnosis of HIV could have, the HIV protection assay is preferably used to confirm the presence of HIV and make an initial determination regarding the suitability of a specific oligonucleotide to treat a patient infected with HIV. Patients determined to be infected with HIV by other assays, but not by the HIV protection assay, should be retested using the HIV protection assay in conjunction with a different oligonucleotide.

## II. DETECTION OF HIV BY OLIGONUCLEOTIDE HYBRIDIZATION

Oligonucleotides targeted to HIV nucleic acids can also be used as detection probes to measure the presence of a HIV target sequence and as amplification primers to selectively amplify HIV nucleic acid. Hybridization to either the target nucleic acid or a nucleotide sequence region complementary to the target sequence is useful for detecting the presence of HIV. Production of nucleic acids having nucleotide sequences complementary to a

target nucleic acid can be obtained using the target nucleic acid as a template in amplification reactions such as polymerase chain reaction (PCR) or transcription mediated amplification methods (e.g., Kacian and Fultz, 5 entitled "Nucleic Acid Amplification Methods," EPO application number 90307503.4.

Useful guidelines for designing probes for HIV detection and amplification primers are described herein and include considerations discussed above, relating to 10 the hybridization of an oligonucleotide to a nucleic acid bearing its complementary sequence. These considerations should be considered in light of the different hybridization conditions under which the oligonucleotides operate. Anti-HIV oligonucleotides are usually used under 15 physiological conditions. In contrast, amplification primers and detection probes can be used under a wider range of conditions, and are preferably used under stringent hybridization assay conditions.

A target nucleotide sequence region present on a 20 nucleic acid molecule is amplified using a primer 5' of the target nucleotide sequence region and a primer 3' of the target nucleotide sequence region. The optimal sites for primer binding are conserved nucleotide sequence regions greater than about 15 bases in length, within 25 about 350 bases, and preferably within 150 bases, of contiguous sequence. Amplification primers are designed to hybridize to these regions. A promoter can be attached to the primer for transcription mediated amplification.

The degree of amplification observed with a set of 30 primers or promotor/primers depend on several factors, including the ability of the oligonucleotides to hybridize to their complementary sequence regions and to be extended enzymatically. Because the extent and specificity of hybridization reactions are affected by several factors, 35 manipulation of those factors determines the exact sensitivity and specificity of a particular oligonucleotide, whether or not it is perfectly

complementary to its target. The importance and effect of various assay conditions are known to those skilled in the art and are described in references such as Hogan et al., EPO Patent Application No. PCT/US87/03009, entitled  
5 "Nucleic Acid Probes for Detection and/or Quantitation of Non-Viral Organisms."

Oligonucleotide detection and amplification probes of a variety of lengths and base compositions may be used, however, preferred probes are between 18 to 100  
10 nucleotides in length, more preferably 18 to 50 nucleotides in length and are sufficiently complementary to the target nucleic acid to hybridize under stringent hybridization conditions (e.g., conditions where probe oligonucleotide hybridizes to an HIV target sequence  
15 region and not to human nucleic acids or nucleic acid from other organisms). Optimal primers have target-binding regions of 18-38 bases, with a predicted  $T_m$  (melting temperature) for the target:primer duplex of about 65°C.

Oligonucleotide detection probes and amplification  
20 primers should be designed to minimize the stability of oligonucleotide:nontarget nucleic acid hybrids. The probes should be able to distinguish between target and non-target nucleotide sequence regions under stringent hybridization conditions. In designing probes, the  
25 differences in  $T_m$  values between oligonucleotide:target and oligonucleotide:non-target duplexes should be as large as possible (e.g., at least 2°C and preferably 5°C).

The secondary structure of the probe and the target region also affects hybridization. Regions of the nucleic  
30 acid forming strong internal structures inhibitory to hybridization are less preferred target sites. Examples of such structures include hairpin stem-loop structures. Likewise, probes with extensive self-complementarity should be avoided. Intramolecular and intermolecular  
35 hybrids can form within a probe if there is sufficient self complementarity. Such structures can be avoided through careful probe design. Commercial computer

programs are available to search for these types of interactions. Available computer programs include MacDNASIS™ 2.0 (Hitachi Software Engineering American Ltd.) and OLIGO® ver. 4.1 (National Bioscience).

5       An integrated genomic target nucleotide sequence region naturally occurs in a double stranded form, as does the product of the polymerase chain reaction (PCR). These double-stranded target nucleic acids inhibit probe:target hybridization. Double stranded target can be accessible  
10 before the hybridization step using standard techniques such as heat denaturation.

The rate of hybridization can be determined by measuring the  $C_0t_{1/2}$ . The rate at which an oligonucleotide hybridizes to its target partly depends on the thermal stability of  
15 the secondary structure of the target nucleic acid in the region complementary to the probe. The standard measurement of hybridization rate is the  $C_0t_{1/2}$ , which is measured as moles of nucleotide per liter times the time in seconds it takes for 50% of the nucleic acids to  
20 hybridize. Thus, the  $C_0t_{1/2}$  is the concentration of free probe times the time at which 50% of maximal hybridization occurs at that concentration. This value is determined by hybridizing various amounts of probe to a constant amount of target for a fixed time. The  $C_0t_{1/2}$  is found graphically  
25 by standard procedure.

The degree of non-specific primer extension (primer-dimer formation or non-target copying) can affect amplification efficiency. Therefore, primers preferably have low self- or cross- complementarity, particularly at  
30 the 3' end. Long homopolymer tracts and high GC content should be avoided to reduce spurious primer extension. Commercial computer programs are available to aid in this aspect of the design. Available computer programs include MacDNASIS™ 2.0 (Hitachi Software Engineering American  
35 Ltd.) and OLIGO® ver. 4.1 (National Bioscience).

Once synthesized, detection probes may be labeled using well known methods (J. Sambrook, E.F. Fritsch and



T. Maniatis, Molecular Cloning, Chapter 11 (2d ed. 1989). Useful labels include fluorescent, chemiluminescent, enzyme and radioactive groups.

### III. SYNTHESIS OF OLIGONUCLEOTIDES

5       Oligonucleotides containing phosphodiester linkages as well as modified linkages can be synthesized by procedures known in the art. For example, Caruthers, et al., In Methods In Enzymology vol. 154 p. 287 (1987), describe a procedure for synthesizing oligonucleotides  
10       containing phosphodiester linkages by standard phosphoramidite solid-phase chemistry; Bhatt, U.S. Patent No. 5,252,723 describes a procedure for synthesizing oligonucleotides containing phosphorothioate linkages; and  
15       Klem et al. entitled "Improved Process for the Synthesis of Oligomers" PCT WO92/07864, describe the synthesis of oligonucleotides having different internucleotide linkages including methylphosphonate linkages.

### IV. EXAMPLES

20       Examples are provided below to illustrate different aspects and embodiments of the present invention. These examples are not intended in any way to limit the disclosed invention.

#### Example 1: Viral Inhibition By Antisense Oligonucleotides

25       This example demonstrates the ability of different antisense oligonucleotides to inhibit HIV replication. The effect of several phosphorothioate antisense oligonucleotides on HIV replication was determined by measuring p24 (an HIV-specific polypeptide) produced by 8e5 cells. The 8e5 cell line is a human T-cell line that  
30       generates replication defective HIV (Folks et al. United States Pat No. 4,752,565). The defective HIV contains a single integrated provirus that expresses all HIV proteins at levels typically observed in chronically infected cell lines except those encoded by *pol*. This defect is due to

a single base insertion that causes a frame shift mutation in *pol*.

Oligonucleotides having phosphorothioate linkages were synthesized according to Bhatt supra. Synthesized  
5 oligonucleotides were purified using HPLC, extracted with ethyl acetate, ethanol precipitated, washed with 80% ethanol and suspended in sterile water to generate high concentration stock solutions that facilitated small volume addition (2-20  $\mu$ l).

10 8e5 cells were cultured in 24 well plates under conditions that resulted in maximal p24 production over the experimental time course. Phosphorothioate antisense oligonucleotides were added directly to 8e5 cultures. Sterile water and non-specific phosphorothioate  
15 oligonucleotides were utilized as controls. The cultures were harvested after 4 days, and the viable cell count and production of p24 antigen were determined. Cell lysates were prepared by spinning down cells from 2 ml wells at 1000 rpm for 10 minutes at 4°C using a Beckman J-10, and  
20 removing the supernatant. The cells were then washed with 5 ml of Hanks balanced salt solution (Gibco) or sterile saline solution (0.9% NaCl), and spun down at 1000 rpm for 10 minutes at 4°C using a Beckman J-10. The cells were resuspended in 300  $\mu$ l of TSM (10 mM Tris-HCl pH 7.5, 150  
25 mM NaCl, 2 mM MgCl<sub>2</sub>) with 0.5 % NP-40, and incubated on ice for 5 minutes. The nuclei and cellular debris were then spun down at 2000 rpm using a Beckman J-10. The amount of p24 antigen was determined in cell lysates using a p24 capture ELISA (American Biotechnologies, Inc.).

30 The ELISA assay for p24 was selected as a measure of HIV replication because expression of *tat* and *rev* are required for efficient p24 production. The percentage of HIV p24 production was calculated as p24/cell production by antisense treated cells divided by p24/cell production  
35 by control treated cells. Control treated cells were treated with a random control phosphorothioate

oligonucleotide (SEQ. ID. NO. 47: AGGCTTCATC ACGTGGACAT TGACG).

As shown in Table 2, several different antisense oligonucleotides inhibited HIV production. The most effective antisense oligonucleotides tested have the nucleic acid sequences SEQ. ID. NOs. 18 or 35. The inhibition with these oligonucleotides ranged from 70-100% (0 to 30% p24 production). The data in Table 2 show the results of a particular experiment. Some of the other antisense oligonucleotides tested caused an increase in the percentage of p24 relative to the control. This phenomena is not well understood.

TABLE 2

INHIBITION OF HIV EXPRESSION USING  
PHOSPHOROTHIOATE ANTI-HIV OLIGONUCLEOTIDES

|    | Oligonucleotide     | Concentration            | % p24             |
|----|---------------------|--------------------------|-------------------|
|    | <u>SEQ. ID. NO.</u> | <u><math>\mu</math>M</u> | <u>production</u> |
|    | 14                  | 2                        | 103               |
|    | 14                  | 10                       | 66                |
| 20 | 17                  | 2                        | 99                |
|    | 17                  | 10                       | 56                |
|    | 18                  | 2                        | 55                |
|    | 18                  | 10                       | 0                 |
|    | 31                  | 2                        | 100               |
| 25 | 31                  | 10                       | 15                |
|    | 33                  | 2                        | 109               |
|    | 33                  | 10                       | 74                |
|    | 35                  | 2                        | 56                |
|    | 35                  | 10                       | 0                 |
| 30 | 36                  | 2                        | 71                |
|    | 36                  | 10                       | 89                |

Several different truncated versions of oligonucleotides having the nucleic acid sequence of SEQ. ID. NOS. 18 or 35 were tested for their ability to inhibit HIV replication. These results are shown in Table 3.

5

TABLE 3

INHIBITION OF HIV EXPRESSION USING SMALL LENGTH  
PHOSPHOROTHIOATE ANTI-HIV OLIGONUCLEOTIDES

|    | Oligonucleotide     | Concentration            | % p24             |
|----|---------------------|--------------------------|-------------------|
|    | <u>SEQ. ID. NO.</u> | <u><math>\mu</math>M</u> | <u>production</u> |
| 10 | 20                  | 10                       | 55                |
|    | 20                  | 20                       | 42                |
|    | 22                  | 10                       | 3                 |
|    | 22                  | 20                       | 31                |
|    | 26                  | 20                       | 44                |
| 15 | 29                  | 10                       | 79                |
|    | 29                  | 20                       | 58                |
|    | 46                  | 10                       | 87                |
|    | 46                  | 20                       | 81                |

- 20 As seen in Table 3, several truncated version of SEQ. ID. NO. 18 (SEQ. ID. NOS. 20, 22, 26, and 29), were particularly effective in inhibiting p24 production. In addition, truncated version of SEQ. ID. NO. 35 (SEQ. ID. NO. 46), was effective in inhibiting p24 production.
- 25 Other appropriate truncated versions can be selected based upon the disclosure provided herein.

Example 2: Viral Inhibition Using Different HIV Strains

- The ability of an antisense oligonucleotide to inhibit HIV replication is shown below using different
- 30 cells infected with different strains of HIV. The nucleic acid sequence of the oligonucleotide used in this experiment is given by SEQ. ID. NO. 18. The nucleotide groups of the oligonucleotide were joined by

phosphorothioate groups and contained deoxyribose moieties.

Specific cells (as described in Table 4) were detached using trypsin-EDTA for 8-10 minutes at 37°C. The  
5 cells were then centrifuged at 100 rpm for 10 minutes. Cells were resuspended in 5-10 ml of maintenance medium (Dulbecco's Modified Eagle Medium ("DMEM") containing 10% fetal calf serum, 500 unites/mg penicillin, 500 units/mg streptomycin, and 2mM glutamine). Cells were maintained  
10 by splitting the culture once weekly into two 75 cm<sup>2</sup> T-flasks at a concentration of  $3 \times 10^4$  cells/ml in a total volume of 200 ml maintenance medium.

On day 0, the cells were plated into the wells of a 24 well tissue culture plate (Falcon #3047) at a  
15 concentration of  $2.4 \times 10^4$  cells per well. The cells were incubated overnight at 37°C. The next day the medium was aspirated, and 200  $\mu$ l of HIV virus at a multiplicity of infection ("MOI") of 0.03 was added to each well. The plates were then incubated for 2 hours at 37°C. After the  
20 2 hour incubation, the cells were treated with 800  $\mu$ l of drug at a concentration (in DMEM) of 1.25x of the final concentration.

The treated cells were incubated for 3 days at 37°C. On day 4 the plate was fixed with 1 ml/well of 100%  
25 methanol for 15 minutes. The wells were then aspirated and 0.5 ml of 0.3% crystal violet stain was added to the wells. After 5 minutes the wells were washed with water and dried. Plaques were counted using a microscope. The number of plaques was plotted versus the concentration of  
30 the antisense oligonucleotide. From the plot IC<sub>50</sub> was determined and the results are presented in Table 4.

TABLE 4  
INHIBITION OF DIFFERENT HIV STRAINS USING THE  
OLIGONUCLEOTIDE OF SEQ. ID. NO. 18

|    | <u>Cell Type</u> | <u>HIV Virus Strain</u>                                      | <u>Drug</u>                                 | <u>Assay</u><br><u>Criterion</u> | <u>IC<sub>50</sub></u> |
|----|------------------|--|---|----------------------------------|------------------------|
| 5  | HeLa-CD4         | HIV-1 (LAI)  | phosphoro-<br>thioate<br>SEQ. ID.<br>NO. 18 | plaques<br>formed                | 200 nM                 |
|    | HeLa-CD4         | HIV-1 (LAI)  | AZT   | plaques<br>formed                | 7-20 nM                |
|    | HeLa-CD4         | Patient Isolates<br>(AO18)<br>AZT Sensitive<br>AZT Resistant | phosphoro-<br>thioate<br>SEQ. ID.<br>NO. 18 | plaques<br>formed                | 80 nM<br>55 nM         |
|    | HeLa-CD4         | Patient Isolates<br>(PO22)<br>AZT Sensitive<br>AZT Resistant | phosphoro-<br>thioate<br>SEQ. ID.<br>NO. 18 | plaques<br>formed                | 45 nM<br>18 nM         |
|    | HeLa-CD4         | HIV-2 (ROD)  | phosphoro-<br>thioate<br>SEQ. ID.<br>NO. 18 | plaques<br>formed                | 18 nM                  |
| 10 | PBMC             | HIV-1 (LAI)  | AZT   | p24                              | 3 nM                   |

|      |             |   |     |        |
|------|-------------|---|-----|--------|
| PBMC | HIV-1 (LAI) | phosphoro-<br>thioate<br>SEQ. ID.<br>NO. 18 | p24 | 120 nM |
|------|-------------|---|-----|--------|

### Example 3: Pharmacological Studies

This example describes pharmacological studies to evaluate the cytotoxicity of anti-HIV oligonucleotides.

5 The nucleic acid sequence of the oligonucleotides used in this example are SEQ. ID. NOs:18 and 22. These oligonucleotides contained phosphorothioate linkages.

The cytotoxicity of the test oligonucleotides was determined in a system using primary human blood cells  
10 using techniques known to those of skill in the art to measure cytotoxicity of anti-neoplastic agents. Human bone marrow cells were harvested and washed in an isotonic solution, then incubated in tissue culture medium containing 0.3% (w/v) agar, 20% (v/v) fetal calf serum,  
15 and 10 ng/ml granulocyte-colony stimulating factor with varying amounts of the two test oligonucleotides. The number of colonies formed were measured after eight days in culture. The IC<sub>50</sub> for colony formation in this experiment was 2-3  $\mu$ M for SEQ. ID. NO:18 and 20-30  $\mu$ M for  
20 SEQ. ID. NO:22.

In vivo experiments were also performed using rodent models. These experiments focused on the potential for gross morphological changes in blood tissue upon  
administration of a phosphorothioate linked  
25 oligonucleotide having the nucleic acid sequence of SEQ. ID. NO:18 (in a solution of PBS). Three mice were given the oligonucleotide at a dose of 100 mg/kg body weight. Four control mice were given PBS alone. Blood samples were taken from each animal at t=0, and at 24 hours each  
30 animal was sacrificed and the organs removed. The

experimental findings with regard to blood parameters are shown in Table 5, and the results obtained upon visual inspection of the organs are shown in Table 6.

TABLE 5

5 EFFECT OF ANTI-HIV OLIGONUCLEOTIDE ON BLOOD PARAMETERS

|    |                                   |                   |                   |
|----|-----------------------------------|-------------------|-------------------|
|    | PBS (n=4)                         | 0 hr              | 24 hr             |
|    | RBC ( $\times 10^4/\mu\text{l}$ ) | 1035.0 $\pm$ 22.4 | 1019.9 $\pm$ 49.7 |
|    | WBC ( $\times 10^2/\mu\text{l}$ ) | 92.5 $\pm$ 19.0   | 82.0 $\pm$ 12.9   |
|    | PLT ( $\times 10^4/\mu\text{l}$ ) | 117.4 $\pm$ 7.6   | 118.3 $\pm$ 7.1   |
| 10 | HGB (g/dl)                        | 16.1 $\pm$ 0.3    | 16.0 $\pm$ 0.6    |
|    | SEQ. ID. NO. 18<br>(n=3)          | 0 hr              | 24 hr             |
|    | RBC ( $\times 10^4/\mu\text{l}$ ) | 1032.3 $\pm$ 8.1  | 990.3 $\pm$ 34.3  |
|    | WBC ( $\times 10^2/\mu\text{l}$ ) | 94.7 $\pm$ 18.2   | 79.3 $\pm$ 15.3   |
| 15 | PLT ( $\times 10^4/\mu\text{l}$ ) | 111.8 $\pm$ 2.7   | 99.4 $\pm$ 0.9    |
|    | HGB (g/dl)                        | 16.0 $\pm$ 0.2    | 15.3 $\pm$ 0.5    |

"RBC" refers to red blood cells. "WBC" refers to white blood cells. "PLT" refers to platelets. "HGB" refers to hemoglobin.



TABLE 6

MORPHOLOGICAL EFFECT OF PHOSPHOROTHIOATE  
LINKED OLIGONUCLEOTIDE OF SEQ. ID. NO. 18

|    |                        |                     |
|----|------------------------|---------------------|
|    | Body Weight            | Unchanged           |
| 5  | Morphological findings |                     |
|    | Bone Marrow            | Normal              |
|    | Liver                  | Normal              |
|    | Kidney                 | Normal              |
|    | Heart                  | Normal              |
| 10 | Thymus                 | Normal              |
|    | Spleen                 | Slight Splenomegaly |

Other embodiments are within the following claims.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

- (A) NAME: GEN-PROBE INCORPORATED
- (B) STREET: 9880 Campus Point Drive
- (C) CITY: San Diego
- (D) STATE: California
- (E) COUNTRY: United States of America
- (F) POSTAL CODE (ZIP): 92121
- (G) TELEPHONE: (619) 546-8000
- (H) TELEFAX: (619) 542-5848

(ii) TITLE OF INVENTION: OLIGONUCLEOTIDES WITH  
ACTIVITY AGAINST  
HUMAN IMMUNODEFI-  
CIENCY VIRUS

(iii) NUMBER OF SEQUENCES: 95

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: WordPerfect 5.1

## (v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: PCT/US94/08407

## (vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: US 08/094,390
- (B) FILING DATE: 19-JUL-1993

## (vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CTTTCAGGTC CCTGTTCGGG CGCCACT

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 27 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CTTTCAAGTC CCTGTTCTGGG CGCCACT 27

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 28 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CTTCTAGCCT CCGCTAGTCA AAATTTTT 28

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 28 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CTTCTAGCCT CCGCTAGTCA AAAATATT 28

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 25 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CTTCTAGCCT CCGCTAGTCA AAATT 25

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(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 28 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CTCCTTCTAG CCTCCGCTAG TCAAAAAT 28

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 28 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CTCTCTCCTT CTAGCCTCCG CTAGTCAA 28

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 27 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TTAATACTGA CGCTCTCGCA CCCATCT 27

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 23 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

ACAACAGACG GGCACACACT AACT 23

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## (2) INFORMATION FOR SEQ ID NO: 10:

## (i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GAGAGAGCTC TGGTTTCCCT TT 22

## (2) INFORMATION FOR SEQ ID NO: 11:

## (i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 26 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

TCGCCGCCCC TGCCTCTTG CCGTGC 26

## (2) INFORMATION FOR SEQ ID NO: 12:

## (i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 26 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

TCCTGCCATA GGAGATGCCT AAGGCC 26

## (2) INFORMATION FOR SEQ ID NO: 13:

## (i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 23 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TTGATGAGTC TGACTGTTCT GAT 23

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(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 20 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

|                      |    |
|----------------------|----|
| TTGGGAGGTG GGTGCTTTG | 20 |
|----------------------|----|

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 26 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

|                              |    |
|------------------------------|----|
| TCTCTCTCTC CACCTTCTTC TTCTAT | 26 |
|------------------------------|----|

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 26 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

|                              |    |
|------------------------------|----|
| TAATCGAATG GATCTGTCTC TGTCTC | 26 |
|------------------------------|----|

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 27 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

|                               |    |
|-------------------------------|----|
| GAGCTCTTCG TCGCTGTCTC CGCTTCT | 27 |
|-------------------------------|----|

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(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 26 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

|                              |    |
|------------------------------|----|
| CTTCGGGCCT GTCGGGTCCC CTCGGG | 26 |
|------------------------------|----|

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 13 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

|                |    |
|----------------|----|
| GCCTGTCGGG TCC | 13 |
|----------------|----|

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 14 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

|                 |    |
|-----------------|----|
| GCCTGTCGGG TCCT | 14 |
|-----------------|----|

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 14 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

|                 |    |
|-----------------|----|
| CGGGTCCCCT CGGG | 14 |
|-----------------|----|

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(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 14 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

|                 |    |
|-----------------|----|
| GCCTGTCGGG TCCC | 14 |
|-----------------|----|

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 14 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

|                 |    |
|-----------------|----|
| CTTCGGGCCT GTCG | 14 |
|-----------------|----|

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 18 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

|                     |    |
|---------------------|----|
| CTTCGGGCCT GTCGGGTC | 18 |
|---------------------|----|

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 18 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

|                     |    |
|---------------------|----|
| GGGCCTGTCG GGTCCCCT | 18 |
|---------------------|----|

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(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 18 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

|                     |    |
|---------------------|----|
| CTGTCGGGTC CCCTCGGG | 18 |
|---------------------|----|

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

|                          |    |
|--------------------------|----|
| TCGGGCCTGT CGGGTCCCCT CG | 22 |
|--------------------------|----|

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

|                          |    |
|--------------------------|----|
| CTTCGGGCCT GTCGGGTCCC CT | 22 |
|--------------------------|----|

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

|                          |    |
|--------------------------|----|
| GGGCCTGTCG GGTCCCCTCG GG | 22 |
|--------------------------|----|

48

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

TAGGATCTAC TGGCTCCATT TC 22

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 23 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

ATTGGTACAA GCAGTTTTAG GCT 23

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 23 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

AGTGGTACAA GCAGTTTTAG GCT 23

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 21 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

GCTTCTTCCT GCCATAGGAG A 21

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(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 24 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

GACTGTTCTG ATGAGCTCTT CGTC 24

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 26 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

GCCTATTCTG CTATGTCGAC ACCCAA 26

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 24 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

CGGGCCTGTC GGGTCCCCTC GGGA 24

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 24 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

CGGGCCTGTC GGGTCCCCTC GGGG 24

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(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 14 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

|                 |    |
|-----------------|----|
| TCTGCTATGT CGAC | 14 |
|-----------------|----|

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 14 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

|                 |    |
|-----------------|----|
| ATGTCGACAC CCAA | 14 |
|-----------------|----|

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 14 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

|                 |    |
|-----------------|----|
| GCCTATTCTG CTAT | 14 |
|-----------------|----|

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 18 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

|                     |    |
|---------------------|----|
| ATTCTGCTAT GTCGACAC | 18 |
|---------------------|----|

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## (2) INFORMATION FOR SEQ ID NO: 42:

## (i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 18 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

TGCTATGTCG ACACCCAA 18

## (2) INFORMATION FOR SEQ ID NO: 43:

## (i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 18 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GCCTATTCTG CTATGTCG 18

## (2) INFORMATION FOR SEQ ID NO: 44:

## (i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

GCCTATTCTG CTATGTCGAC AC 22

## (2) INFORMATION FOR SEQ ID NO: 45:

## (i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

CTATTCTGCT ATGTCGACAC CC 22

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(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

ATTCTGCTAT GTCGACACCC AA 22

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 25 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

AGGCTTCATC ACGTGGACAT TGACG 25

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 27 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

CUUUCAGGUC CCUGUUCGGG CGCCACU 27

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 27 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

CUUUCAAGUC CCUGUUCGGG CGCCACU 27

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(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 28 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

CUUCUAGCCU CCGCUAGUCA AAAUUUUU 28

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 28 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

CUUCUAGCCU CCGCUAGUCA AAAUAUU 28

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 26 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

CAUUUCCUAU CUUAGCGUUU CUUCCC 26

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 28 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

CUCCUUCUAG CCUCCGCUAG UCAAAAAU 28

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(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 28 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

CUCUCUCCUU CUAGCCUCCG CUAGUCAA 28

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 27 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

UUA AUACUGA CGCUCUCGCA CCAUCU 27

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 23 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

ACAACAGACG GGCACACACU AACU 23

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

GAGAGAGCUC UGGUUUCCCU UU 22



55

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

|                         |    |
|-------------------------|----|
| GUCAUGUAU GUCCAAGUUG GC | 22 |
|-------------------------|----|

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 26 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

|                              |    |
|------------------------------|----|
| UCCUGCCAUA GGAGAUGCCU AAGGCC | 26 |
|------------------------------|----|

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 23 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

|                          |    |
|--------------------------|----|
| UGAUGAGUC UGACUGUUCU GAU | 23 |
|--------------------------|----|

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 20 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

|                       |    |
|-----------------------|----|
| UUGGGAGGUG GGUUGCUUUG | 20 |
|-----------------------|----|

56

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 26 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

UCUCUCUCUC CACCUUCUUC UUCUAU 26

(2) INFORMATION FOR SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 26 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

UAAUCGAAUG GAUCUGUCUC UGUCUC 26

(2) INFORMATION FOR SEQ ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 27 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

GAGCUCUUCG UGCUGUCUC CGCUUCU 27

(2) INFORMATION FOR SEQ ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 26 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

CUUCGGGCCU GUCGGGUCCC CUCGGG 26

57

(2) INFORMATION FOR SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 13 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

GCCUGUCGGG UCC 13

(2) INFORMATION FOR SEQ ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 14 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

GCCUGUCGGG UCCU 14

(2) INFORMATION FOR SEQ ID NO: 68:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 14 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

CGGGUCCCCU CGGG 14

(2) INFORMATION FOR SEQ ID NO: 69:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 14 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

GCCUGUCGGG UCCC 14

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(2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 14 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

CUUCGGGCCU GUCG 14

(2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 18 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

CUUCGGGCCU GUCGGGUC 18

(2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 18 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

GGGCCUGUCG GGUCCCCU 18

(2) INFORMATION FOR SEQ ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 18 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

CUGUCGGGUC CCCUCGGG 18

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## (2) INFORMATION FOR SEQ ID NO: 74:

## (i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

UCGGGCCUGU CGGGUCCCCU CG 22

## (2) INFORMATION FOR SEQ ID NO: 75:

## (i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

CUUCGGGCCU GUCGGGUCCC CU 22

## (2) INFORMATION FOR SEQ ID NO: 76:

## (i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 76:

GGGCCUGUCG GGUCCCCUCG GG 22

## (2) INFORMATION FOR SEQ ID NO: 77:

## (i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 77:

UAGGAUCUAC UGGCUCCAUC UC 22

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(2) INFORMATION FOR SEQ ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 23 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 78:

AUUGGUACAA GCAGUUUUAG GCU 23

(2) INFORMATION FOR SEQ ID NO: 79:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 23 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 79:

AGUGGUACAA GCAGUUUUAG GCU 23

(2) INFORMATION FOR SEQ ID NO: 80:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 21 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 80:

GCUUCUCCU GCCAUAGGAG A 21

(2) INFORMATION FOR SEQ ID NO: 81:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 24 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 81:

GACUGUUCUG AUGAGCUCU CGUC 24

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(2) INFORMATION FOR SEQ ID NO: 82:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 26 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 82:

|                              |    |
|------------------------------|----|
| GCCUAUUCUG CUAUGUCGAC ACCCAA | 26 |
|------------------------------|----|

(2) INFORMATION FOR SEQ ID NO: 83:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 24 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 83:

|                            |    |
|----------------------------|----|
| CGGGCCUGUC GGGUCCCCUC GGGA | 24 |
|----------------------------|----|

(2) INFORMATION FOR SEQ ID NO: 84:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 24 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 84:

|                            |    |
|----------------------------|----|
| CGGGCCUGUC GGGUCCCCUC GGGG | 24 |
|----------------------------|----|

(2) INFORMATION FOR SEQ ID NO: 85:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 14 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 85:

|                 |    |
|-----------------|----|
| UCUGCUAUGU CGAC | 14 |
|-----------------|----|

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(2) INFORMATION FOR SEQ ID NO: 86:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 14 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 86:

AUGUCGACAC CCAA 14

(2) INFORMATION FOR SEQ ID NO: 87:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 14 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 87:

GCCUAUUCUG CUAU 14

(2) INFORMATION FOR SEQ ID NO: 88:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 18 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 88:

AUUCUGCUAU GUCGACAC 18

(2) INFORMATION FOR SEQ ID NO: 89:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 18 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 89:

UGCUAUGUCG ACACCCAA 18



63

(2) INFORMATION FOR SEQ ID NO: 90:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 18 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 90:

|                     |    |
|---------------------|----|
| GCCUAUUCUG CUAUGUCG | 18 |
|---------------------|----|

(2) INFORMATION FOR SEQ ID NO: 91:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 91:

|                          |    |
|--------------------------|----|
| GCCUAUUCUG CUAUGUCGAC AC | 22 |
|--------------------------|----|

(2) INFORMATION FOR SEQ ID NO: 92:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 92:

|                          |    |
|--------------------------|----|
| CUAUUCUGCU AUGUCGACAC CC | 22 |
|--------------------------|----|

(2) INFORMATION FOR SEQ ID NO: 93:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 22 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 93:

|                          |    |
|--------------------------|----|
| AUUCUGCUAU GUCGACACCC AA | 22 |
|--------------------------|----|

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(2) INFORMATION FOR SEQ ID NO: 94:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 26 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 94:

UUGGGUGUCG ACAUAGCAGA AUAGGC 26

(2) INFORMATION FOR SEQ ID NO: 95:

(i) SEQUENCE CHARACTERISTICS:

|                   |               |
|-------------------|---------------|
| (A) LENGTH:       | 26 base pairs |
| (B) TYPE:         | nucleic acid  |
| (C) STRANDEDNESS: | single        |
| (D) TOPOLOGY:     | linear        |

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 95:

CCCGAGGGGA CCCGACAGGC CCGAAG 26

CLAIMS

1. A method of inhibiting HIV replication comprising the step of contacting cells infected with HIV with a purified oligonucleotide 20 to 100 nucleotides in  
5 length, said oligonucleotide substantially complementary to a nucleic acid sequence region of 20 nucleotides present in an HIV nucleic acid sequence selected from the group consisting of:  
SEQ ID NO: 94: UUGGGUGUCG ACAUAGCAGA AUAGGC, and  
10 SEQ ID NO: 95: CCCGAGGGGA CCCGACAGGC CCGAAG,  
wherein said oligonucleotide inhibits HIV replication *in vivo* or *in vitro*.

2. The method of claim 1, wherein said oligonucleotide comprises at least one phosphorothioate  
15 linkage.

3. A method of inhibiting HIV replication comprising the use of a purified oligonucleotide consisting essentially of a nucleotide sequence region selected from the group consisting of:  
20 SEQ ID NO: 18: CTTGCGGCCT GTCGGGTCCC CTCGGG,  
SEQ ID NO: 19: GCCTGTCGGG TCC,  
SEQ ID NO: 20: GCCTGTCGGG TCCT,  
SEQ ID NO: 22: GCCTGTCGGG TCCC,  
SEQ ID NO: 23: CTTGCGGCCT GTCG,  
25 SEQ ID NO: 24: CTTGCGGCCT GTCGGGTC,  
SEQ ID NO: 25: GGGCCTGTCG GGTCCCCT,  
SEQ ID NO: 26: CTGTCGGGTC CCCTCGGG,  
SEQ ID NO: 27: TCGGGCCTGT CGGGTCCCCT CG,  
SEQ ID NO: 28: CTTGCGGCCT GTCGGGTCCC CT,  
30 SEQ ID NO: 29: GGGCCTGTCG GGTCCCCTCG GG,  
SEQ ID NO: 35: GCCTATTCTG CTATGTCGAC ACCCAA,  
SEQ ID NO: 38: TCTGCTATGT CGAC,  
SEQ ID NO: 39: ATGTCGACAC CCAA,  
SEQ ID NO: 40: GCCTATTCTG CTAT,

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SEQ ID NO: 41: ATTCTGCTAT GTCGACAC,  
 SEQ ID NO: 42: TGCTATGTCG ACACCCAA,  
 SEQ ID NO: 43: GCCTATTCTG CTATGTCG,  
 SEQ ID NO: 44: GCCTATTCTG CTATGTCGAC AC,  
 5 SEQ ID NO: 45: CTATTCTGCT ATGTCGACAC CC,  
 SEQ ID NO: 46: ATTCTGCTAT GTCGACACCC AA,  
 SEQ ID NO: 65: CUUCGGGCCU GUCGGGUCCC CUCGGG,  
 SEQ ID NO: 66: GCCUGUCGGG UCC,  
 SEQ ID NO: 67: GCCUGUCGGG UCCU,  
 10 SEQ ID NO: 69: GCCUGUCGGG UCCC,  
 SEQ ID NO: 70: CUUCGGGCCU GUCG,  
 SEQ ID NO: 71: CUUCGGGCCU GUCGGGUC,  
 SEQ ID NO: 72: GGGCCUGUCG GGUCCCCU,  
 SEQ ID NO: 73: CUGUCGGGUC CCCUCGGG,  
 15 SEQ ID NO: 74: UCGGGCCUGU CGGGUCCCCU CG,  
 SEQ ID NO: 75: CUUCGGGCCU GUCGGGUCCC CU,  
 SEQ ID NO: 76: GGGCCUGUCG GGUCCCCUCG GG,  
 SEQ ID NO: 82: GCCUAUUCUG CUAUGUCGAC ACCCAA  
 SEQ ID NO: 85: UCUGCUAUGU CGAC,  
 20 SEQ ID NO: 86: AUGUCGACAC CCAA,  
 SEQ ID NO: 87: GCCUAUUCUG CUAU,  
 SEQ ID NO: 88: AUUCUGCUAU GUCGACAC,  
 SEQ ID NO: 89: UGCUAUGUCG ACACCCAA,  
 SEQ ID NO: 90: GCCUAUUCUG CUAUGUCG,  
 25 SEQ ID NO: 91: GCCUAUUCUG CUAUGUCGAC AC,  
 SEQ ID NO: 92: CUAUUCUGCU AUGUCGACAC CC, and  
 SEQ ID NO: 93: AUUCUGCUAU GUCGACACCC AA,  
 wherein said oligonucleotide inhibits HIV replication in  
 vivo or in vitro.

30        4.    The method of claim 3, wherein said  
 oligonucleotide is predominantly comprised of  
 phosphorothioate linkages.

5.    The method of claim 4, wherein said nucleic acid  
 sequence region is selected from the group consisting of:

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SEQ ID NO: 18: CTTCGGGCCT GTCGGGTCCC CTCGGG,  
 SEQ ID NO: 20: GCCTGTCGGG TCCT,  
 SEQ ID NO: 22: GCCTGTCGGG TCCC,  
 SEQ ID NO: 26: CTGTCGGGTC CCCTCGGG,  
 5 SEQ ID NO: 29: GGGCCTGTCG GGTCCCCTCG GG,  
 SEQ ID NO: 35: GCCTATTCTG CTATGTCGAC ACCCAA,  
 SEQ ID NO: 65: CUUCGGGCCU GUCGGGUCCC CUCGGG,  
 SEQ ID NO: 67: GCCUGUCGGG UCCU,  
 SEQ ID NO: 69: GCCUGUCGGG UCCC,  
 10 SEQ ID NO: 73: CUGUCGGGUC CCCUCGGG,  
 SEQ ID NO: 76: GGGCCUGUCG GGUCCCCUCG GG, and  
 SEQ ID NO: 82: GCCUAUUCUG CUAUGUCGAC ACCCAA.

6. The method of claim 5, wherein said  
 oligonucleotide consisting of the nucleotide sequence  
 15 region

SEQ ID NO: 18: CTTCGGGCCT GTCGGGTCCC CTCGGG.

7. A method for treatment of a patient infected  
 with HIV comprising the step of administering to said  
 patient a purified oligonucleotide consisting essentially  
 20 of a nucleotide sequence region selected from the group  
 consisting of:

SEQ ID NO: 18: CTTCGGGCCT GTCGGGTCCC CTCGGG,  
 SEQ ID NO: 19: GCCTGTCGGG TCC,  
 SEQ ID NO: 20: GCCTGTCGGG TCCT,  
 25 SEQ ID NO: 22: GCCTGTCGGG TCCC,  
 SEQ ID NO: 23: CTTCGGGCCT GTCG,  
 SEQ ID NO: 24: CTTCGGGCCT GTCGGGTC,  
 SEQ ID NO: 25: GGGCCTGTCG GGTCCCCT,  
 SEQ ID NO: 26: CTGTCGGGTC CCCTCGGG,  
 30 SEQ ID NO: 27: TCGGGCCTGT CGGGTCCCCT CG,  
 SEQ ID NO: 28: CTTCGGGCCT GTCGGGTCCC CT,  
 SEQ ID NO: 29: GGGCCTGTCG GGTCCCCTCG GG,  
 SEQ ID NO: 35: GCCTATTCTG CTATGTCGAC ACCCAA,  
 SEQ ID NO: 38: TCTGCTATGT CGAC,  
 35 SEQ ID NO: 39: ATGTCGACAC CCAA,

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SEQ ID NO: 40: GCCTATTCTG CTAT,  
 SEQ ID NO: 41: ATTCTGCTAT GTCGACAC,  
 SEQ ID NO: 42: TGCTATGTCG ACACCCAA,  
 SEQ ID NO: 43: GCCTATTCTG CTATGTCG,  
 5 SEQ ID NO: 44: GCCTATTCTG CTATGTCGAC AC,  
 SEQ ID NO: 45: CTATTCTGCT ATGTCGACAC CC,  
 SEQ ID NO: 46: ATTCTGCTAT GTCGACACCC AA,  
 SEQ ID NO: 65: CUUCGGGCCU GUCGGGUCCC CUCGGG,  
 SEQ ID NO: 66: GCCUGUCGGG UCC,  
 10 SEQ ID NO: 67: GCCUGUCGGG UCCU,  
 SEQ ID NO: 69: GCCUGUCGGG UCCC,  
 SEQ ID NO: 70: CUUCGGGCCU GUCG,  
 SEQ ID NO: 71: CUUCGGGCCU GUCGGGUC,  
 SEQ ID NO: 72: GGGCCUGUCG GGUCCCCU,  
 15 SEQ ID NO: 73: CUGUCGGGUC CCCUCGGG,  
 SEQ ID NO: 74: UCGGGCCUGU CGGGUCCCCU CG,  
 SEQ ID NO: 75: CUUCGGGCCU GUCGGGUCCC CU,  
 SEQ ID NO: 76: GGGCCUGUCG GGUCCCCUCG GG,  
 SEQ ID NO: 82: GCCUAUUCUG CUAUGUCGAC ACCCAA  
 20 SEQ ID NO: 85: UCUGCUAUGU CGAC,  
 SEQ ID NO: 86: AUGUCGACAC CCAA,  
 SEQ ID NO: 87: GCCUAUUCUG CUAU,  
 SEQ ID NO: 88: AUUCUGCUAU GUCGACAC,  
 SEQ ID NO: 89: UGCUAUGUCG ACACCCAA;  
 25 SEQ ID NO: 90: GCCUAUUCUG CUAUGUCG,  
 SEQ ID NO: 91: GCCUAUUCUG CUAUGUCGAC AC,  
 SEQ ID NO: 92: CUAUUCUGCU AUGUCGACAC CC, and  
 SEQ ID NO: 93: AUUCUGCUAU GUCGACACCC AA,  
 wherein said oligonucleotide inhibits HIV replication in  
 30 vivo or *in vitro*.

8. The method of claim 7, wherein said oligonucleotide is predominantly comprised of phosphorothioate linkages.

9. The method of claim 8, wherein said nucleic acid  
 35 sequence region is selected from the group consisting of:

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SEQ ID NO: 18: CTCGGGCCT GTCGGGTCCC CTCGGG,  
SEQ ID NO: 20: GCCTGTCGGG TCCT,  
SEQ ID NO: 22: GCCTGTCGGG TCCC,  
SEQ ID NO: 26: CTGTCGGGTC CCCTCGGG,  
5 SEQ ID NO: 29: GGGCCTGTCG GGTCCCCTCG GG,  
SEQ ID NO: 35: GCCTATTCTG CTATGTCGAC ACCCAA,  
SEQ ID NO: 65: CUUCGGGCCU GUCGGGUCCC CUCGGG,  
SEQ ID NO: 67: GCCUGUCGGG UCCU,  
SEQ ID NO: 69: GCCUGUCGGG UCCC,  
10 SEQ ID NO: 73: CUGUCGGGUC CCCUCGGG,  
SEQ ID NO: 76: GGGCCUGUCG GGUCCCCUCG GG, and  
SEQ ID NO: 82: GCCUAUUCUG CUAUGUCGAC ACCCAA.

10. The method of claim 9, wherein said  
oligonucleotide consisting of the nucleotide sequence  
15 region of

SEQ ID NO: 18: CTCGGGCCT GTCGGGTCCC CTCGGG.

11. A method for treatment of a patient infected  
with HIV comprising the step of administering to said  
patient a purified oligonucleotide having a nucleotide  
20 sequence region selected from the group consisting of:

SEQ ID NO: 18: CTCGGGCCT GTCGGGTCCC CTCGGG,  
SEQ ID NO: 19: GCCTGTCGGG TCC,  
SEQ ID NO: 20: GCCTGTCGGG TCCT,  
SEQ ID NO: 22: GCCTGTCGGG TCCC,  
25 SEQ ID NO: 23: CTCGGGCCT GTCG,  
SEQ ID NO: 24: CTCGGGCCT GTCGGGTC,  
SEQ ID NO: 25: GGGCCTGTCG GGTCCCCT,  
SEQ ID NO: 26: CTGTCGGGTC CCCTCGGG,  
SEQ ID NO: 27: TCGGGCCTGT CGGGTCCCCT CG,  
30 SEQ ID NO: 28: CTCGGGCCT GTCGGGTCCC CT,  
SEQ ID NO: 29: GGGCCTGTCG GGTCCCCTCG GG,  
SEQ ID NO: 35: GCCTATTCTG CTATGTCGAC ACCCAA,  
SEQ ID NO: 38: TCTGCTATGT CGAC,  
SEQ ID NO: 39: ATGTCGACAC CCAA,  
35 SEQ ID NO: 40: GCCTATTCTG CTAT,

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SEQ ID NO: 41: ATTCTGCTAT GTCGACAC,  
 SEQ ID NO: 42: TGCTATGTCG ACACCCAA,  
 SEQ ID NO: 43: GCCTATTCTG CTATGTCG,  
 SEQ ID NO: 44: GCCTATTCTG CTATGTCGAC AC,  
 5 SEQ ID NO: 45: CTATTCTGCT ATGTCGACAC CC,  
 SEQ ID NO: 46: ATTCTGCTAT GTCGACACCC AA,  
 SEQ ID NO: 65: CUUCGGGCCU GUCGGGUCCC CUCGGG,  
 SEQ ID NO: 66: GCCUGUCGGG UCC,  
 SEQ ID NO: 67: GCCUGUCGGG UCCU,  
 10 SEQ ID NO: 69: GCCUGUCGGG UCCC,  
 SEQ ID NO: 70: CUUCGGGCCU GUCG,  
 SEQ ID NO: 71: CUUCGGGCCU GUCGGGUC,  
 SEQ ID NO: 72: GGGCCUGUCG GGUCCCCU,  
 SEQ ID NO: 73: CUGUCGGGUC CCCUCGGG,  
 15 SEQ ID NO: 74: UCGGGCCUGU CGGGUCCCCU CG,  
 SEQ ID NO: 75: CUUCGGGCCU GUCGGGUCCC CU,  
 SEQ ID NO: 76: GGGCCUGUCG GGUCCCCUCG GG,  
 SEQ ID NO: 82: GCCUAUUCUG CUAUGUCGAC ACCCAA  
 SEQ ID NO: 85: UCUGCUAUGU CGAC,  
 20 SEQ ID NO: 86: AUGUCGACAC CCAA,  
 SEQ ID NO: 87: GCCUAUUCUG CUAU,  
 SEQ ID NO: 88: AUUCUGCUAU GUCGACAC,  
 SEQ ID NO: 89: UGCUAUGUCG ACACCCAA,  
 SEQ ID NO: 90: GCCUAUUCUG CUAUGUCG,  
 25 SEQ ID NO: 91: GCCUAUUCUG CUAUGUCGAC AC,  
 SEQ ID NO: 92: CUAUUCUGCU AUGUCGACAC CC, and  
 SEQ ID NO: 93: AUUCUGCUAU GUCGACACCC AA,  
 wherein said oligonucleotide inhibits HIV replication *in vivo* or *in vitro*.

30        12. The method of claim 11, wherein said  
 oligonucleotide is predominantly comprised of  
 phosphorothioate linkages.



13. The method of claim 12, wherein said nucleic acid sequence region is selected from the group consisting of:

- SEQ ID NO: 18: CTCGGGCCT GTCGGGTCCC CTCGGG,  
5 SEQ ID NO: 20: GCCTGTCGGG TCCT,  
SEQ ID NO: 22: GCCTGTCGGG TCCC,  
SEQ ID NO: 26: CTGTCGGGTC CCCTCGGG,  
SEQ ID NO: 29: GGGCCTGTCG GGTCCCCTCG GG,  
SEQ ID NO: 35: GCCTATTCTG CTATGTCGAC ACCCAA,  
10 SEQ ID NO: 65: CUUCGGGCCU GUCGGGUCCC CUCGGG,  
SEQ ID NO: 67: GCCUGUCGGG UCCU,  
SEQ ID NO: 69: GCCUGUCGGG UCCC,  
SEQ ID NO: 73: CUGUCGGGUC CCCUCGGG,  
SEQ ID NO: 76: GGGCCUGUCG GGUCCCCUCG GG, and  
15 SEQ ID NO: 82: GCCUAUUCUG CUAUGUCGAC ACCCAA.

14. An oligonucleotide targeted to HIV nucleic acid comprising a purified oligonucleotide 20 to 100 nucleotides substantially complementary to a nucleic acid sequence region of 20 nucleotides present in an HIV  
20 nucleic acid sequence selected from the group consisting of:

- SEQ ID NO: 94: UUGGGUGUCG ACAUAGCAGA AUAGGC, and  
SEQ ID NO: 95: CCCGAGGGGA CCCGACAGGC CCGAAG,  
wherein said oligonucleotide inhibits HIV replication *in vivo* or *in vitro*.  
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15. The oligonucleotide of claim 14 wherein said oligonucleotide comprises at least one phosphorothioate linkage.

16. An oligonucleotide consisting essentially of a  
30 nucleotide sequence region selected from the group consisting of:

- SEQ ID NO: 18: CTCGGGCCT GTCGGGTCCC CTCGGG,  
SEQ ID NO: 19: GCCTGTCGGG TCC,  
SEQ ID NO: 20: GCCTGTCGGG TCCT,

SEQ ID NO: 22: GCCTGTCGGG TCCC,  
SEQ ID NO: 23: CTTCGGGCCT GTCG,  
SEQ ID NO: 24: CTTCGGGCCT GTCGGGTC,  
SEQ ID NO: 25: GGGCCTGTCG GGTCCCCT,  
5 SEQ ID NO: 26: CTGTCGGGTC CCCTCGGG,  
SEQ ID NO: 27: TCGGGCCTGT CGGGTCCCCT CG,  
SEQ ID NO: 28: CTTCGGGCCT GTCGGGTCCC CT,  
SEQ ID NO: 29: GGGCCTGTCG GGTCCCCTCG GG,  
SEQ ID NO: 35: GCCTATTCTG CTATGTCGAC ACCCAA,  
10 SEQ ID NO: 38: TCTGCTATGT CGAC,  
SEQ ID NO: 39: ATGTCGACAC CCAA,  
SEQ ID NO: 40: GCCTATTCTG CTAT,  
SEQ ID NO: 41: ATTCTGCTAT GTCGACAC,  
SEQ ID NO: 42: TGCTATGTCG ACACCCAA,  
15 SEQ ID NO: 43: GCCTATTCTG CTATGTCG,  
SEQ ID NO: 44: GCCTATTCTG CTATGTCGAC AC,  
SEQ ID NO: 45: CTATTCTGCT ATGTCGACAC CC,  
SEQ ID NO: 46: ATTCTGCTAT GTCGACACCC AA,  
SEQ ID NO: 65: CUUCGGGCCU GUCGGGUCCC CUCGGG,  
20 SEQ ID NO: 66: GCCUGUCGGG UCC,  
SEQ ID NO: 67: GCCUGUCGGG UCCU,  
SEQ ID NO: 69: GCCUGUCGGG UCCC,  
SEQ ID NO: 70: CUUCGGGCCU GUCG,  
SEQ ID NO: 71: CUUCGGGCCU GUCGGGUC,  
25 SEQ ID NO: 72: GGGCCUGUCG GGUCCCCU,  
SEQ ID NO: 73: CUGUCGGGUC CCCUCGGG,  
SEQ ID NO: 74: UCGGGCCUGU CGGGUCCCCU CG,  
SEQ ID NO: 75: CUUCGGGCCU GUCGGGUCCC CU,  
SEQ ID NO: 76: GGGCCUGUCG GGUCCCCUCG GG,  
30 SEQ ID NO: 82: GCCUAUUCUG CUAUGUCGAC ACCCAA  
SEQ ID NO: 85: UCUGCUAUGU CGAC,  
SEQ ID NO: 86: AUGUCGACAC CCAA,  
SEQ ID NO: 87: GCCUAUUCUG CUAU,  
SEQ ID NO: 88: AUUCUGCUAU GUCGACAC,  
35 SEQ ID NO: 89: UGCUAUGUCG ACACCCAA,  
SEQ ID NO: 90: GCCUAUUCUG CUAUGUCG,  
SEQ ID NO: 91: GCCUAUUCUG CUAUGUCGAC AC,

SEQ ID NO: 92: CUAUUCUGCU AUGUCGACAC CC, and  
SEQ ID NO: 93: AUUCUGCUAU GUCGACACCC AA,  
wherein said oligonucleotide inhibits HIV replication *in vivo* or *in vitro*.

- 5        17. The oligonucleotide of claim 16, wherein said oligonucleotide is predominantly comprised of phosphorothioate linkages.

18. The oligonucleotide of claim 17, wherein said nucleic acid sequence region is selected from the group  
10 consisting of:

SEQ ID NO: 18: CTCGGGCCT GTCGGGTCCC CTCGGG,  
SEQ ID NO: 20: GCCTGTCGGG TCCT,  
SEQ ID NO: 22: GCCTGTCGGG TCCC,  
SEQ ID NO: 26: CTGTCGGGTC CCCTCGGG,  
15 SEQ ID NO: 29: GGGCCTGTCG GGTCCCCTCG GG,  
SEQ ID NO: 35: GCCTATTCTG CTATGTCGAC ACCCAA,  
SEQ ID NO: 65: CUUCGGGCCU GUCGGGUCCC CUCGGG,  
SEQ ID NO: 67: GCCUGUCGGG UCCU,  
SEQ ID NO: 69: GCCUGUCGGG UCCC,  
20 SEQ ID NO: 73: CUGUCGGGUC CCCUCGGG,  
SEQ ID NO: 76: GGGCCUGUCG GGUCCCCUCG GG, and  
SEQ ID NO: 82: GCCUAUUCUG CUAUGUCGAC ACCCAA.

19. The oligonucleotide of claim 18 consisting of  
SEQ ID NO: 18: CTCGGGCCT GTCGGGTCCC CTCGGG.

- 25        20. A purified oligonucleotide having a nucleotide sequence region selected from the group consisting of:

SEQ ID NO: 18: CTCGGGCCT GTCGGGTCCC CTCGGG,  
SEQ ID NO: 19: GCCTGTCGGG TCC,  
SEQ ID NO: 20: GCCTGTCGGG TCCT,  
30 SEQ ID NO: 22: GCCTGTCGGG TCCC,  
SEQ ID NO: 23: CTCGGGCCT GTCG,  
SEQ ID NO: 24: CTCGGGCCT GTCGGGTC,  
SEQ ID NO: 25: GGGCCTGTCG GGTCCCCT,

SEQ ID NO: 26: CTGTCGGGTC CCCTCGGG,  
 SEQ ID NO: 27: TCGGGCCTGT CGGGTCCCCT CG,  
 SEQ ID NO: 28: CTCGGGCCT GTCGGGTCCC CT,  
 SEQ ID NO: 29: GGGCCTGTCG GGTCCCCTCG GG,  
 5 SEQ ID NO: 35: GCCTATTCTG CTATGTCGAC ACCCAA,  
 SEQ ID NO: 38: TCTGCTATGT CGAC,  
 SEQ ID NO: 39: ATGTCGACAC CCAA,  
 SEQ ID NO: 40: GCCTATTCTG CTAT,  
 SEQ ID NO: 41: ATTCTGCTAT GTCGACAC,  
 10 SEQ ID NO: 42: TGCTATGTCG ACACCCAA,  
 SEQ ID NO: 43: GCCTATTCTG CTATGTCG,  
 SEQ ID NO: 44: GCCTATTCTG CTATGTCGAC AC,  
 SEQ ID NO: 45: CTATTCTGCT ATGTCGACAC CC,  
 SEQ ID NO: 46: ATTCTGCTAT GTCGACACCC AA,  
 15 SEQ ID NO: 65: CUUCGGGCCU GUCGGGUCCC CUCGGG,  
 SEQ ID NO: 66: GCCUGUCGGG UCC,  
 SEQ ID NO: 67: GCCUGUCGGG UCCU,  
 SEQ ID NO: 69: GCCUGUCGGG UCCC,  
 SEQ ID NO: 70: CUUCGGGCCU GUCG,  
 20 SEQ ID NO: 71: CUUCGGGCCU GUCGGGUC,  
 SEQ ID NO: 72: GGGCCUGUCG GGUCCCCU,  
 SEQ ID NO: 73: CUGUCGGGUC CCCUCGGG,  
 SEQ ID NO: 74: UCGGGCCUGU CGGGUCCCCU CG,  
 SEQ ID NO: 75: CUUCGGGCCU GUCGGGUCCC CU,  
 25 SEQ ID NO: 76: GGGCCUGUCG GGUCCCCUCG GG,  
 SEQ ID NO: 82: GCCUAUUCUG CUAUGUCGAC ACCCAA  
 SEQ ID NO: 85: UCUGCUAUGU CGAC,  
 SEQ ID NO: 86: AUGUCGACAC CCAA,  
 SEQ ID NO: 87: GCCUAUUCUG CUAU,  
 30 SEQ ID NO: 88: AUUCUGCUAU GUCGACAC,  
 SEQ ID NO: 89: UGCUAUGUCG ACACCCAA,  
 SEQ ID NO: 90: GCCUAUUCUG CUAUGUCG,  
 SEQ ID NO: 91: GCCUAUUCUG CUAUGUCGAC AC,  
 SEQ ID NO: 92: CUAUUCUGCU AUGUCGACAC CC, and  
 35 SEQ ID NO: 93: AUUCUGCUAU GUCGACACCC AA,  
 wherein said oligonucleotide inhibits HIV replication *in vivo* or *in vitro*.

21. The oligonucleotide of claim 20, wherein said oligonucleotide is predominantly comprised of phosphorothioate linkages.

22. The oligonucleotide of claim 21, wherein said  
5 nucleic acid sequence region is selected from the group consisting of:
- SEQ ID NO: 18: CTCGGGCCT GTCGGGTCCC CTCGGG,  
SEQ ID NO: 20: GCCTGTCGGG TCCT,  
SEQ ID NO: 22: GCCTGTCGGG TCCC,  
10 SEQ ID NO: 26: CTGTCGGGTC CCCTCGGG,  
SEQ ID NO: 29: GGGCCTGTCG GGTCCCCTCG GG,  
SEQ ID NO: 35: GCCTATTCTG CTATGTCGAC ACCCAA,  
SEQ ID NO: 65: CUUCGGGCCU GUCGGGUCCC CUCGGG,  
SEQ ID NO: 67: GCCUGUCGGG UCCU,  
15 SEQ ID NO: 69: GCCUGUCGGG UCCC,  
SEQ ID NO: 73: CUGUCGGGUC CCCUCGGG,  
SEQ ID NO: 76: GGGCCUGUCG GGUCCCCUCG GG, and  
SEQ ID NO: 82: GCCUAUUCUG CUAUGUCGAC ACCCAA.